THE AMERICAN OYSTER

CRASSOSTREA VIRGINICA Gmelin

by PAUL S. GALTSOFF

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Crassostrea virginica Gmelin

By PAUL S. GALTSOFF

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Created in 1849, the United States Department of the Interior—a department of conservation—is concerned with the management, conservation, and development of the Nation's water, fish, wildlife, mineral, forest, and park and recreational resources. It also has major responsibilities for Indian and Territorial affairs.

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PREFACE

The book on American oysters, *Crassostrea virginica*, of the Atlantic and Gulf States has been written for biologists, administrators of oyster resources of various States, public health officers, students of marine biology, and oyster growers who may be interested to learn about the life history and mode of living of this species. Trying to be scientifically accurate and at the same time to make the text understandable to those who have only elementary knowledge of biological sciences, I have attempted to present the facts and discuss various theories in the simplest language. Scientific terminology and the origin of terms has been explained wherever in my opinion the expression was not generally known to the reader. Various techniques used in anatomical, histological, and physiological studies have been described in detail. I hope the information provided in the text is sufficient for anyone who desires to engage in oyster research to apply the described techniques.

Each of the 18 chapters comprising the book can be read independently of others. The reader not familiar with oyster anatomy is advised, however, to read first chapter IV which gives a general description of the principal systems of organs. Selected bibliographical references accompany each chapter. In the course of preparing the text for publication a very large bibliography on oysters and related subjects was prepared and deposited in the library of the Bureau of Commercial Fisheries Biological Laboratory, Woods Hole, Mass. The file comprises over 6,000 subject and author cards.

The completion of the book would have been impossible without the assistance of Patricia A. Philpott, who took meticulous care in verifying all bibliographic references by comparing them with the originals; was responsible for final arrangement of illustrations and checking the captions; and several times retyped the revised text. Her interest, secretarial competence, and initiative are gratefully acknowledged.

The work on the bibliography could not have been accomplished without the extensive use of the library of the Marine Biological Laboratory, Woods Hole, Mass., and the valuable cooperation of the librarian, Mrs. Deborah Harlow.

My personal gratitude is due to many of my associates in the Government Service for their cooperation in field and laboratory work, and to the scientists who at my request were kind enough to read the chapters of the book within the scope of their respective specialization. Their valuable comments were taken into consideration. Histological preparations for chapters V to XIV inclusive were made by Eugenia Galtsoff, whose voluntary service is greatly appreciated.

I acknowledge with thanks the comments on the text made by the following persons: W. R. Amberson, M. R. Carriker, D. P. Costello, R. L. Edwards, Catherine Henley, E. Higgins, B. Ketchum, Marie B. Lambert, F. L. Lambert, A. S. Merrill, X. Musacchia, H. Rehder, G. A. Rounsefell, H. B. Stenzel, and C. G. Wilber. Ann Martin was kind enough to edit the text from the point of view of clearness and style.

Credit for the majority of the illustrations is due to Ruth Von Arx whose artistic ability combined with a background in biology were instrumental in producing clear and accurate pictures of various structures.

Most of the experimental research work was made during the long period of my association with the Bureau of Commercial Fisheries Biological Laboratory, and the Marine Biological Laboratory, at Woods Hole, Mass. Additional laboratory studies were conducted at the Bureau of Commercial Fisheries Biological Laboratories at Milford, Conn., Beaufort, N.C., Pensacola, Fla., and at the Marine Laboratory of Stanford University, Pacific Grove, Calif.

During the period of nearly 40 years I had an opportunity to make occasional field surveys and conduct ecological studies on oyster bottoms of every coastal state of the United States, in the Hawaiian Islands, in the Gulf of Panama, and on Margarita Island, Venezuela. All field work was conducted with the valuable cooperation of local state governments.

Paul S. Galtsoff

Woods Hole, Mass.

THE AMERICAN OYSTER *CRASSOSTREA VIRGINICA* GMELIN

By Paul S. Galtsoff, *Fishery Biologist*
Bureau of Commercial Fisheries

CHAPTER I

TAXONOMY

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The family Ostreidae consists of a large number of edible and nonedible oysters. Their distribution is confined to a broad belt of coastal waters within the latitudes 64° N. and 44° S. With few exceptions oysters thrive in shallow water, their vertical distribution extending from a level approximately halfway between high and low tide levels to a depth of about 100 feet. Commercially exploited oyster beds are rarely found below a depth of 40 feet.

The name "Ostrea" was given by Linnaeus (1758) to a number of mollusks which he described as follows:

"Ostrea. Animal Tethys, testa bivalvis inaequivalvis, subaurita. Cardo edentulus and fossula cava ovata, striisque lateralibus transversis. Vulva anusve nullus." The name Tethys (from Greek mythology and also refers to the sea) applies to the type of marine animals, living either within the shells or naked, that Linnaeus listed under a general name "Vermes" which includes worms, mollusks, echinoderms, and others. The translation of Linnaeus' diagnosis reads as follows: Shell bivalve, unequal, almost ear-shaped. Hinge toothless, depression concave and oval-shaped, with transverse lines on the sides. No vulva or anus.

This broad characterization included a number of genera such as scallops, pen shells (Pinnidae), limas (Limidae) and other mollusks which obviously are not oysters. In the 10th edition of "Systema Naturae," Linnaeus (1758) wrote:

"Ostreae non omnes, imprimis Pectines, ad cardinem interne fulcis transversis numerosis parallelis in utraque testa oppositis gaudentiquae probe distinguendae ab *Arcis polypleptoginglymis*, cujus dentes numerosi alternatim intrant alterius sinus." i.e., not all are oysters, in particular the scallops, which have many parallel ribs running crosswise inward toward the hinge on each shell on opposite sides; these should properly be distinguished from *Arca polyleptoginglymis* whose many teeth alternately enter between the teeth of the other side.

In the same publication the European flat oyster, *Ostrea edulis*, is described as follows: "Vulgo *Ostrea* dictae *edulis*. *O. testa* semiorbiculata membranis imbricatis undulatis: valvula altera plana integerrima." i.e., commonly called edible oyster; shell semicircular, outer valve with wavy grooves; the other small valve completely flat. With a minor change Linnaeus' diagnosis is repeated by Gmelin (1789).

Lamarck (1801) restricted the family Ostreidae to the species of the genus *Ostrea* which was characterized by him as follows: Adhering shell, valves unequal, irregular, with divergent beaks which with age become very unequal; the upper valve becomes misplaced. Hinge without teeth. Ligament half internal, inserted in the cardinal lunule of the valves; the lunule of the lower valve and the beak grow with age and sometimes reach great length.

Great confusion in the usage of the generic name of living oysters resulted, however, from

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Note.—Approved for publication April 24, 1964.

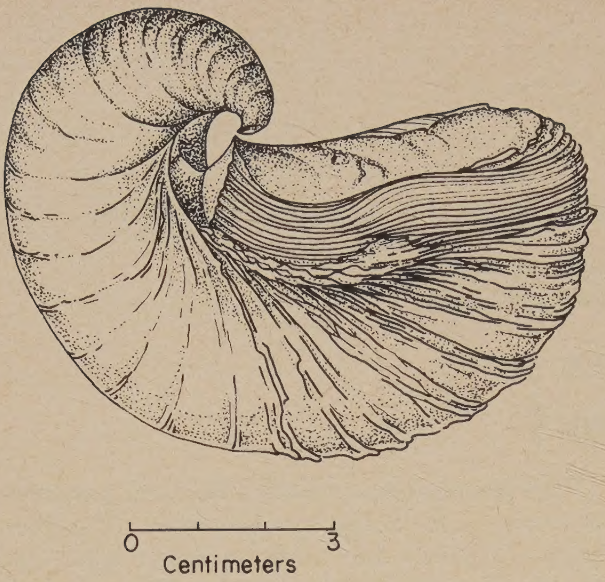

FIGURE 1.—*Gryphaea arcuata*, Jurassic fossil. Specimen No. 283 from the Museum of Comparative Zoology, Harvard University. Dimensions: height 8.5 cm., depth 3.5 cm.

Lamarck's (1801) allocation of extinct and recent species of oysters to the genus *Gryphaea*. Under this generic name, which was published with a diagnosis, Lamarck included nine nominal species, some of which at the time of publication were "nomina nuda" since they lacked diagnosis, but other species were validated by citation of bibliographical references (Hemming, 1951). Lamarck had not designated the type species, and a selection of "types" was made by Anton (1839), who designated fossil *Gryphaea arcuata* (fig. 1) as type. Dall (1898) and Anthony (1906) also selected *G. arcuata* as type. A living species, *Gryphaea angulata*, was included by Lamarck (1801) but without a diagnosis.

Lamarck (1819) further confused the nomenclature of the genus *Ostrea* when he again described the genus *Gryphaea*. A longer list of species included common oyster of Portugal and Mediterranean, *Gryphaea angulata*, this time, however, with a diagnosis (fig. 2). It was assumed by Children, Grey, Fisher, Tryon, Stoliczka, and Sacco (quoted from Dall, 1898: 671–688) that *G. angulata* is Lamarck's type species, an opinion entirely without foundation. Anatomical and embryological studies have demonstrated that *G. angulata* has no characteristics of the fossils *Gryphaeidae*. The species is simply another type of oyster similar to the American species (*C. virginica*), with a slightly twisted beak which only remotely resembles the curved beak of the *Gryphaea*.

Oysters are frequently found so closely adhering to the substratum that their shells faithfully reproduce the configurations and detailed structures of the objects upon which they rest. For instance, under the name of *Ostrea tuberculata*, Lamarck (1819) described a shell from the Timor Sea (Netherland Indies) grown on a coral of the family Astraeidae; this particular shell repeated the tubercles and other structural elements of the coral upon which it was resting. Other specimens of the same species, but grown on a smooth surface, were listed as different species. *O. haliotidea* of Australia, another Lamarckian species assumed a shape of the abalone shell to which it was attached. Oysters adhering to the shells of *Trochus maculatus* repeated the granular structure of this gastropod (Smith, 1878), while those grown on branches of mangrove trees usually formed a groove between the folds of the shell facing the branch while the same species attached to the trunk of the tree did not develop such structure (Gray, 1833). *O. equestris* found growing on navigation buoys (Galtsoff and Merrill, 1962) repeated the configurations of bolts and shells of barnacles upon which they happened to attach themselves.

The influence of other factors of the environment on the shape and sculpture of oyster shell has been reported by many investigators who noticed that specimens growing in calm water on flat surfaces have a tendency to acquire a round shape and to have poorly developed umbones. On soft bottom and overcrowded reefs the same species tend to form long and slender, laterally compressed bodies with hooklike umbones. Lamy (1929) observed that oysters attached to a pebble or shell and, therefore, slightly raised above the bottom, had deep lower valves, more or less radially ribbed. This type of structure, according to Lamy, offered greater resistance to dislodgment by currents or wave action.

Since Lamarck's inclusion of a living estuarine species, *G. angulata*, in one genus with the fossil Jurassic and Cretaceous Gryphaeas was not acceptable to many biologists, the question was submitted for ruling by the International Commission on Zoological Nomenclature. The retention of the name "*Gryphaea*" and the designation of *G. angulata* as the type species of the genus *Gryphaea* was favored by the "majority" of European zool-

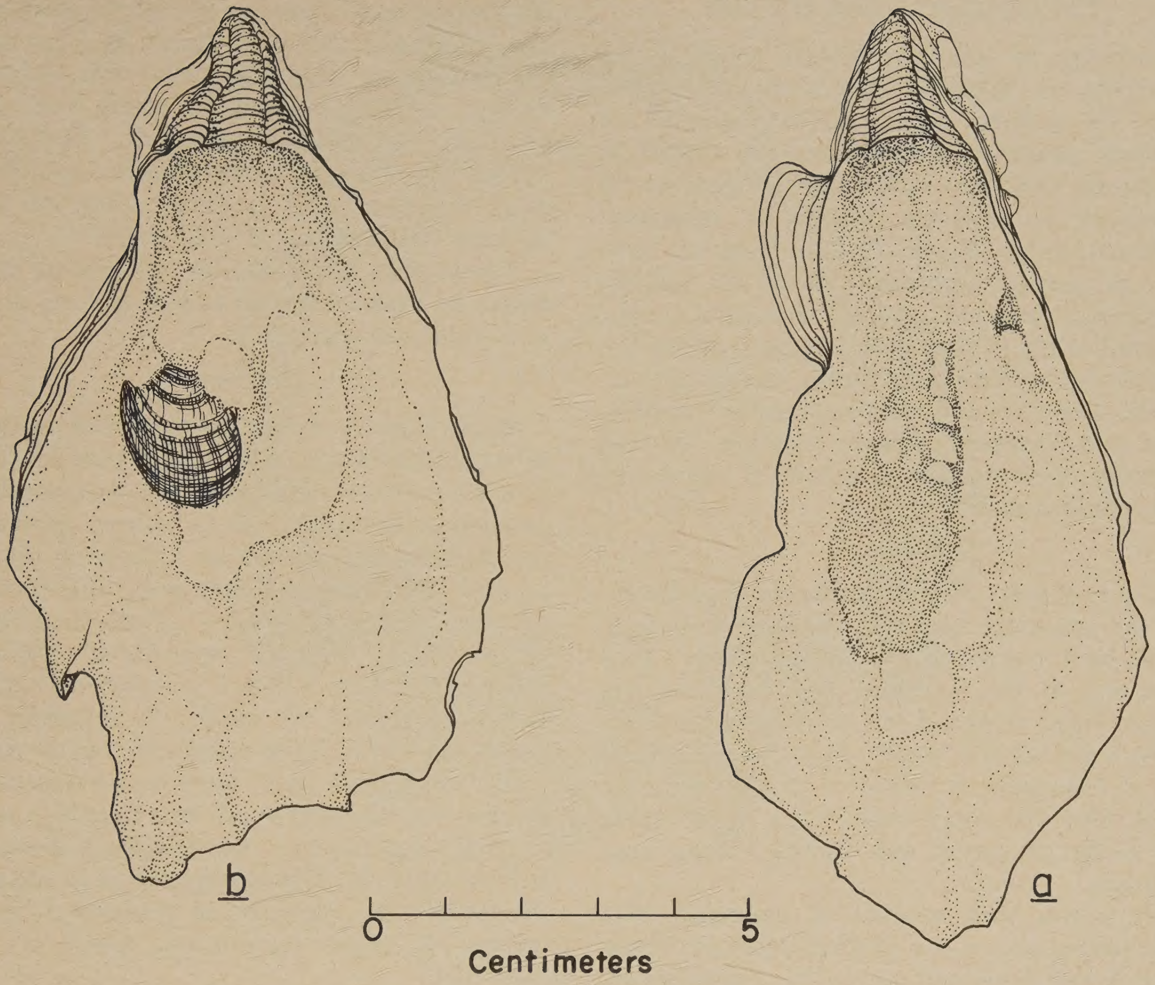

Figure 2.—(a) *Crassostrea angulata,* Arcachon, France. Dimensions: height 9 cm., length 5 cm. (b) *C. virginica,* Brownsville, Tex. Dimensions: height 8.5 cm., length 4.5 cm. Note similarity of the two forms.

ogists (Ranson, 1948a) who requested the International Commission to suppress the name "*Gryphaea*" (Lamarck 1801) as applied to fossil species and to validate the name "*Gryphaea*" (Lamarck 1819) which included the living oysters. The American zoologists (Gunter, 1950) were in favor of retaining the name "*Gryphaea*" for fossil forms.

The findings of the International Commission, published as Opinion 338 on March 17, 1955, are as follows:

Gryphaea Lamarck, 1801, is available for the purposes of the Law of Priority and has as its type species the Mesozoic Fossil species *G. arcuata* Lamarck, 1801, by selection by Anton (1839), and not *G. angulata* Lamarck, 1801, which was selected by Children in 1823, this latter name being a nomen nudum (not having been published with an indication for the recent species to which it is applicable until 1819).

"This nominal species *Gryphaea angulata* Lamarck, 1819, is not the type species of any nominal genus, but the generic name *Crassostrea* Sacco, 1897, is available for use for that species by those specialists who regard it as congeneric with *Ostrea virginica* Gmelin, (1790) (the type species of *Crassostrea* Sacco) and who do not refer both species to the genus *Ostrea* Linnaeus, 1758."

The names *Gryphaea* Lamarck, 1801, *Crassostrea* Sacco, 1897, *arcuata* Lamarck, 1801 (*Gryphaea*) and *angulata* Lamarck, 1819 (*Gryphaea*) were placed on the Official Lists of Generic Names and Specific Names respectively and the nomen nudum angulata Lamarck, 1801 (*Grypnaea*) was placed on the Official Index of Rejected and Invalid Names in Zoology.

TAXONOMIC CHARACTERS

According to the view shared today by all specialists on pelecypod taxonomy, the genus *Ostrea* (in a broad sense), as characterized by Lamarck, comprises several groups of oysters of the family Ostreidae sufficiently different to be considered as separate genera or subgenera (Lamarck, 1819; Thiele, 1935). There is, however, no general agreement about the validity of various genera and species. A uniform system of classification of oysters is lacking, and for the separation of genera and species various authors use the characters of different categories: namely, shape and structure of shell, anatomy, sex and spawning, habitat, and structure of the larval shell (prodissoconch).

SHELL

In spite of great variability, certain shell characters are generally constant although they may be obscured in grossly distorted specimens. Two characters of this category are important: the cavity of the valves and the structure of the shell.

The lower valve is usually deep and cup-shaped with a depression or recess of greater or lesser extent near the hinge area. The upper valve may be flat or curved and slightly bulging near the hinge.

The oyster shell consists of extremely thin outer periostracum, the median prismatic layer, which is well developed on the flat (right) valve, the inner calcitic ostracum that constitutes the major part of the shell thickness, and hypostracum, a very thin layer of aragonite (orthorhombic $CaCO_3$) pad under the place of attachment of the adductor muscle.[1] The prismatic layer of *Ostrea edulis* is confined to the intricate brown scales of the flat valve, while among the Australian oysters only one species, *O. angasi* Sowerby, has a well-developed prismatic layer (Thomson, 1954). In the genus *Pycnodonte* the shell is peculiarly vacuolated (Ranson, 1941). The white patches or so-called "chalky deposits" in the shells of many oysters are not significant as taxonomic characters.

Size, shape, curvature, and proportion of the beak, i.e., the pointed (dorsal) end of an oyster shell, are useful generic characters, but like other parts of the shell they are variable and cannot be entirely depended upon for identification.

The sculpture of the shell may be useful for recognizing some species (*O.* (*Alectryonia*) *megodon*, fig. 3) with valves reinforced by a number of prominences or folds (also called ribs, ridges, or flutings by various authors) which end in the crenulations at the edge of the shell. In American oysters this character varies greatly depending on local conditions but is rather constant in *O. equestris* (Galtsoff and Merrill, 1962).

The position of the muscle scar and its outline differs in various species and, therefore, is used as a taxonomic character.

ANATOMY

Anatomical characters are of limited usefulness to malacologists who have to base their identification primarily on shells alone. Consequently the anatomical characteristics have been ignored by the majority of taxonomists. Some of the anatomical differences are, however, important for the separation of the genera. Thus, the presence of the promyal chamber separates the *Ostrea*, in which this feature is absent in sufficiently studied species, *O. edulis*, *O. lurida*, *O. equestris*, from *Crassostrea* in which the chamber is well developed.

Size of the gill ostia, large in the larviparous species and relatively small in oviparous ones, is of generic significance. The relation of the rectum and the heart is of importance since in the genus *Pycnodonte* the ventricle is penetrated by the rectum, a unique feature not found in other Ostreidae.

Convolution of the edge of the mantle with three folds in the majority of the species and only two in some Japanese species (Hirase, 1930) has been mentioned by some investigators as a specific character. The existence of two or three folds may be significant, but other characters such as ridges of the mantle, pigmentation of the tentacles and their size and spacing are variable and, in my opinion, have no taxonomic value.

SEX AND SPAWNING

On the basis of sexual habits, oysters fall into two distinct categories of nonincubatory (or oviparous) species, (*Crassostrea* spp.) i.e., those in which the eggs discharged into the water are fertilized outside the organism; and the incubatory or larviparous species (*Ostrea* spp.) in which fertilization takes place in the gill cavity, and the larvae are incubated and discharged after having reached an advanced stage of development. Incubatory oysters, as for example *O. edulis*, *O. lurida*, and *O. equestris*, are bisexual

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[1] The author is grateful to H. B. Stenzel for the information on aragonite in oyster shells.

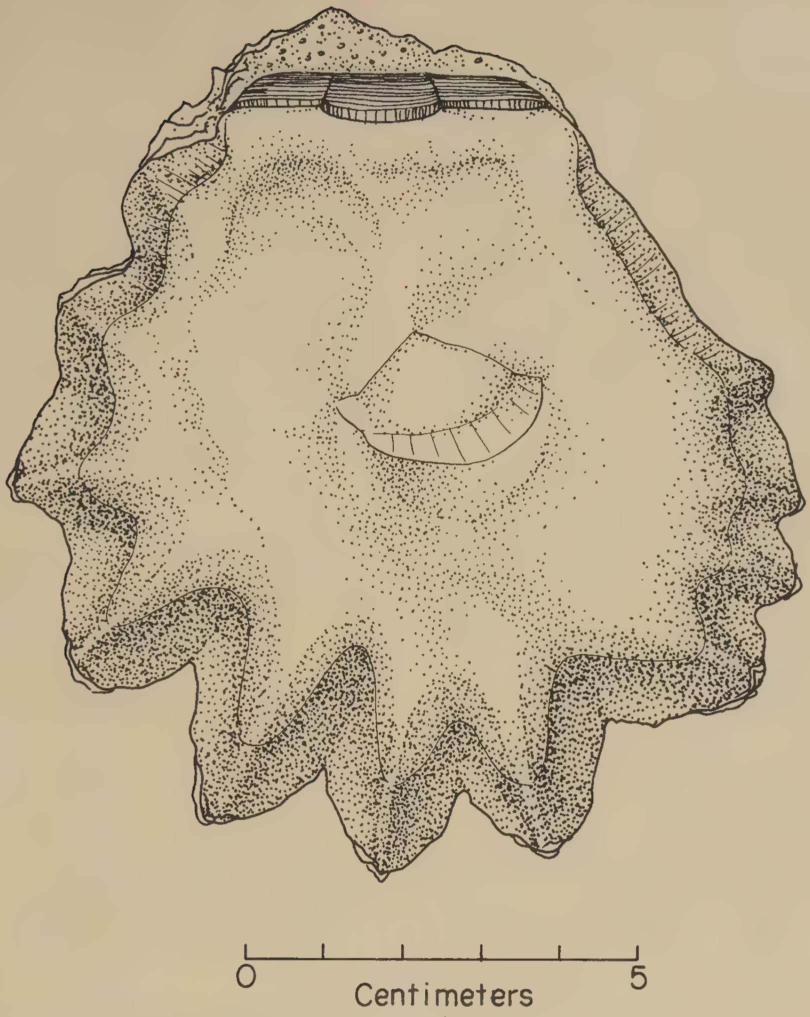

FIGURE 3.—*O.* (*Alectryonia*) *megodon*. Pearl Islands, Panama. Dimensions: height 17 cm., length 16 cm.

(hermaphroditic). The gonad of a bisexual oyster produces eggs and sperm simultaneously, but the relative quantity of sex cells of one or another type alternates periodically from male to female and vice versa.

The sexes of nonincubatory (oviparous) oysters (*C. virginica*, *C. gigas*, *C. angulata*) are separate. Instances of hermaphroditism in this group are very rare. The sexes are, however, unstable and once a year a certain percentage of oysters change their sex. This change takes place after spawning during the indifferent phase of gonad development. Alternation of sex (discussed in ch. XV) has been studied in detail only for a few species.

HABITAT

Salinity, turbidity, and depth of water are frequently mentioned in the brief statements that accompany the descriptions of various species. Ecological data are, however, of no help to classification. With the exception of a few commercial species, which have been more adequately studied, little is known about the environmental requirements of the populations of other species. In a general way it can be stated that *C. virginica*, *C. gigas*, and probably *C. angulata* are more tolerant to diluted sea water than are *O. lurida* and *O. edulis*. The two latter species survive better in more saline and less turbid environment. Geographical distribution of *O. equestris* suggests the preference of this species to the waters of full oceanic salinity (about 35°/oo). The same is true for many tropical oysters living along the continental shores and in the lagoons of oceanic islands where the salinity of water changes but little or remains constant throughout the year. Tolerances of these tropical species to lowered salinity have not been studied.

LARVAL SHELL (PRODISSOCONCH)

The difficulty in identifying a species by its shell led Ranson (1948b) to base the classification of oysters entirely on the features of a "definite" prodissoconch, i.e., the shell of a fully developed larva. He claims that distinctive crenulations of prodissoconchs are sufficient for the separation of species and that these specifically larval characters can be detected in well-preserved adult shells and even in fossils. In a brief paper comprising only 6 incomplete pages of text and 35 pages of drawings of 34 species of oyster larvae Ranson (1960) summarizes the basic idea of his classification. He states that a lamellibranch larval shell passes through two distinct stages, the first one is a "primitive" prodissoconch with undifferentiated hinge and the second phase, which he calls "definite" prodissoconch, is characterized by the development of hinge teeth. At the first phase all lamellibranchs have similar prodissoconchs, but at the second phase the hinge becomes differentiated. This makes it possible to distinguish the families and the genera. He maintains, without giving substantiating evidence, that the general shape of a definite prodissoconch is absolutely constant even if the size of the larva varies and that each species of oysters can be recognized by the shape of its larval shell and its structural characteristics.

Ranson's system of classification recognizes the following three genera of oysters: *Pycnodonte* (*Pycnodonta* in Ranson's spelling), *Crassostrea*, and *Ostrea*. His diagnoses of the prodissoconchs of these genera are given in the following section of this chapter. Unfortunately, the diagnoses of the larvae of 34 species of oysters studied by Ranson are lacking, and the prodissoconchs are shown only by diagrammatic drawings, some of which are reproduced in chapter XVI of the book. My attempts to locate the prodissoconchs on the shells of fully grown *C. virginica* (from 3 to 8 years old) were not successful. On a few occasions the prodissoconchs were faintly visible, but the structure of the hinge, and the number and location of hinge teeth could not be detected. Final decision regarding the possibility of identifying adult oysters by their larval characters must wait, however, until Ranson's system is given a fair trial by malacologists. His suggestion that identification can be made by observing spat attached to the shell of the adult is not valid because in many places several species of oysters live together in the same locality and the larvae settle indiscriminately on any shell or other object available at the time of setting.

THE GENERA OF LIVING OYSTERS

There is an obvious need for a complete taxonomic revision of the family Ostreidae. This revision should cover all the principal species of living oysters and must be supplemented by morphological, anatomical, and ecological observations which at present are available only for a few commercially utilized species. In the absence of these data for the large majority of species of living oysters, it is at present impossible to propose a logical taxonomical system for the family.

Opinions vary regarding the number of genera of living oysters of the family Ostreidae. Stenzel (1947) recognizes 12 valid generic names, some of which, as was shown by Gunter (1950), are synonymous. The latter author admits the existence of three definite genera (*Ostrea*, *Crassostrea* and *Pycnodonte*) and three others (*Dendostrea* Swainson, *Alectryonia* Fisher de Waldheim, and *Striostrea* Vialov) of doubtful validity.

Ranson (1941, 1948b, 1960) merges the three doubtful genera of Gunter in *Ostrea* and recognizes only the three definite genera listed above. This opinion, based primarily on structure of prodissoconchs, is shared by Thomson (1954) and is supported by the evidence accumulating from morphological and biological data. It can be, therefore, stated with a certain degree of assurance that on the basis of present knowledge, the living Ostreidae comprise three genera: namely, *Ostrea* Linnaeus; *Crassostrea* Sacco; and *Pycnodonte* Fisher de Waldheim. The genus *Lopha*, named by Bolton in 1798, without definition and described as a subgenus only in 1898 by Dall (1898), is undoubtedly a synonym of *Ostrea*. The distinctive feature upon which this genus was founded was the sharply crenulated nature of the shell margin, a very poor distinguishing character.

The three genera of oysters can be defined as follows:

Genus *Ostrea* Linné, 1758. Genotype: *O. edulis* L.

Shell subcircular; lower valve shallow, not recessed under the hinge; upper valve flat, opercular, sometimes domed; muscle scar subcentral. Promyal chamber absent. Gill ostia relatively large. Incubatory.

Prodissoconch with long hinge; two denticles at each end, the anterior pair frequently reduced; the ligament is internal at the level of the hinge, at the center, and between the center and anterior end (Ranson, 1960, fig. 350, ch. XVI).

Genus *Crassostrea* Sacco, 1897. Genotype: *C. virginica* Gmelin

Shell very variable, usually elongated; lower valve cuplike, deep, and recessed under the hinge; upper valve flat, opercular. Muscle scar displaced in dorsolateral direction. Large promyal chamber on the right side of the body. Gill ostia and eggs relatively small. Nonincubatory. Excellent illustrations of the species can be found in the monographs of Lister (1685) and Chemnitz (1785).

This genus includes species formerly known as *O. virginica*, *O. gigas*, and *G. angulata*. The separation of the cuplike oysters (*Crassostrea*) from the flat ones (*Ostrea*) is justified because of the anatomical differences (promyal chamber, size of the ostia) and spawning habits. The name "*Crassostrea*" (Sacco, 1897) is validated in accordance with the rules of the International Commission on Zoological Nomenclature (1955).

Valves of the prodissoconch unequal; hinge with two teeth at each end; internal anterior ligament extends beyond the hinge (Ranson, 1960). (Fig. 348, ch. XVI).

Genus *Pynodonte* Fisher de Waldheim, 1835. Genotype: *P. radiata* F. de W.

Shells large and heavy; lower valve slightly recessed under hinge; both shells lack sculpture except for sharp crenulations along the tip; hinge very broad. Inner sides of valves chalky white or greenish; row of small denticles along the edges of valves on both sides of the hinge; muscle scar white, elevated on a shelflike projection; the adductor muscle is oval in outline and rounded on the hinge side; the gonads are bright orange; the ventricle of the heart surrounds the rectum, which runs posteriorly beyond the adductor muscle almost to the junction of the mantle. Nonincubatory.

Valves of prodissoconch equal; hinge with five teeth arranged over the entire length of it; internal anterior ligament, immediately after the hinge; 10 small denticles at the edge of each valve anteriorly to the ligament (Ranson, 1960).

The following species of living oysters are known from the coastal waters of the continental United States and from the State of Hawaii:

C. virginica Gmelin, Eastern oyster, Atlantic oyster. This is the principal edible oyster of the Atlantic and Gulf Coasts of the U.S.A. (fig. 4). Its range of distribution extends from the Gulf of St. Lawrence to the Gulf of Mexico and the West Indies. The species was introduced in the waters of San Francisco Bay, Puget Sound, Willapa Bay, and Oahu Island but failed to establish itself, although occasionally single specimens can be found in these waters.

The right (upper) valve smaller than the left. The beaks elongated and strongly curved. The valve margins straight or only slightly undulating. The muscle scar usually deeply pigmented. The adductor muscle located asymmetrically, well toward the posterodistal border. Large promyal chamber on the right side. Nonincubatory, discharging eggs and sperm directly into the water.

Adults vary from 2 to 14 inches in height (dorsoventral direction) depending on age and environment. Shape, sculpture, and pigmentation of inner side of the shell and along the edges of the mantle and tentacles vary greatly.

Crassostrea rhizophorae Guilding. Light, thin, foliaceous, and deeply cupped shell with smaller flat upper valve fitting to the lower one (fig. 5). The inner margins straight and smooth with considerable purple coloration especially around the left valve. The beaks twisted dorsally. Muscle scar near the dorsal margin. Promyal chamber present. Nonincubatory, discharging eggs and sperm directly into the water.

Similar to *C. virginica* from which it differs by the following characters: lower left valve is less plicated than in *virginica;* the muscle scar is more rounded and often unpigmented. (Prodissoconch shown in fig. 349, ch. XVI).

Adults may reach 4 inches in height. Frequently attached to the aerial roots of the mangrove *Rhizophora mangle*. Inhabits Caribbean region including Puerto Rico and Cuba where the species is commercially exploited.

Crassostrea gigas Thunberg, Japanese oyster, Pacific oyster (fig. 6) Cuplike shells of large size with coarse and widely spaced concentric lamellae and coarse ridges on the outside; shells usually much thinner than those of *C. virginica*. Upper (right) valve flat and smaller than lower (left)

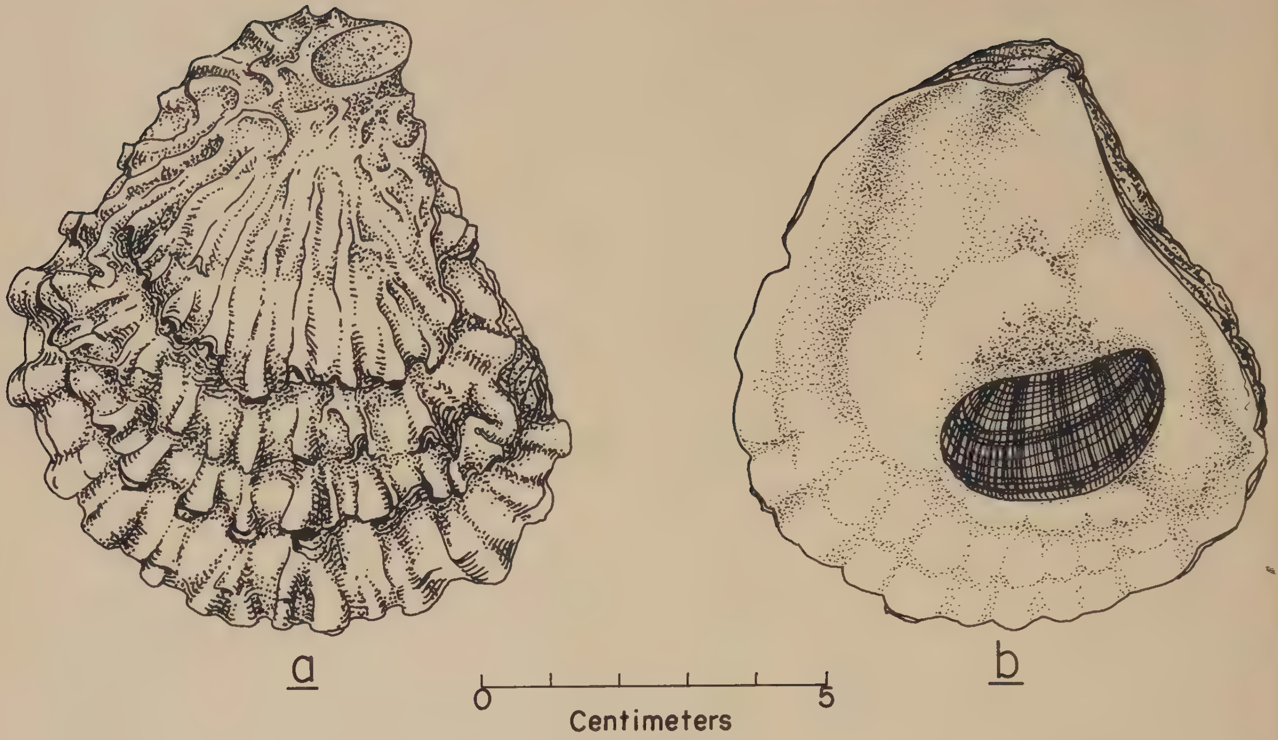

FIGURE 4.—*C. virginica.* Wellfleet Harbor, Mass. Dimensions: height 9.5 cm., length 8 cm. (a) lower (left) valve; (b) inside of upper (right) valve.

valve. Interior surface white, often with faint purplish stain over the muscle scar or near the edges. Large promyal chamber on the right side. Edges of the mantle deeply pigmented. Nonincubatory, discharging a very large number of eggs and sperm directly into the water. Introduced from Japan into the waters of British Columbia, western states of the United States, and Alaska (Ketchikan). Small number of specimens of *C. gigas* were at various times planted in Mobile Bay, Ala., and in Barnstable Bay, Mass.

Highly variable. Typical *C. gigas* is a long, straplike oyster. The form *C. laperousi* (considered by Japanese malacologists as a separate species) has round, highly ridged shells.

C. commercialis (Iredale and Roughley), Sydney rock oyster, commercial oyster. This Australian species (fig. 7) was imported to Hawaii about 1925–28 and planted along the shores of the western end of Kaneohe Bay, in Oahu Island. In 1930 several of the imported specimens were examined by the author and found to be ripe and spawning. During World War II the small population of this species was destroyed by dredging operations.

Valves markedly unequal and variable. The left valve deep and cup-shaped, recessed under the hinge, slightly fluted; the edge weakly crenulated. The upper valve flattened. Inner side of valves chalky white, frequently with bluish or creamy markings on the upper valve; muscle scar usually not pigmented. Edges of valves with small denticles extending about half way around the valve. Sexes are separate; nonincubatory.

Usually grows to 3 to 4 inches in height, but cultivated specimens have been reported to reach 10-inch size. Normal range of distribution New South Wales and Queensland; frequently found in the intertidal zone attached to rocks, sticks, and shells.

C. rivularis Gould (fig. 8). This Japanese species has been planted in waters of Puget Sound with the shipments of seed of *C. gigas*. In Japan the oyster is known as "suminoegaki" (Hirase, 1930).

The shell orbicular; strong and large, adult specimens reaching 6 inches in height. Left, lower, valve slightly concave, upper valve shorter and flat. The left valve with generally indistinct lamellae of pale pink color with radiating striae. The lamellae of the right valve are thin and al-

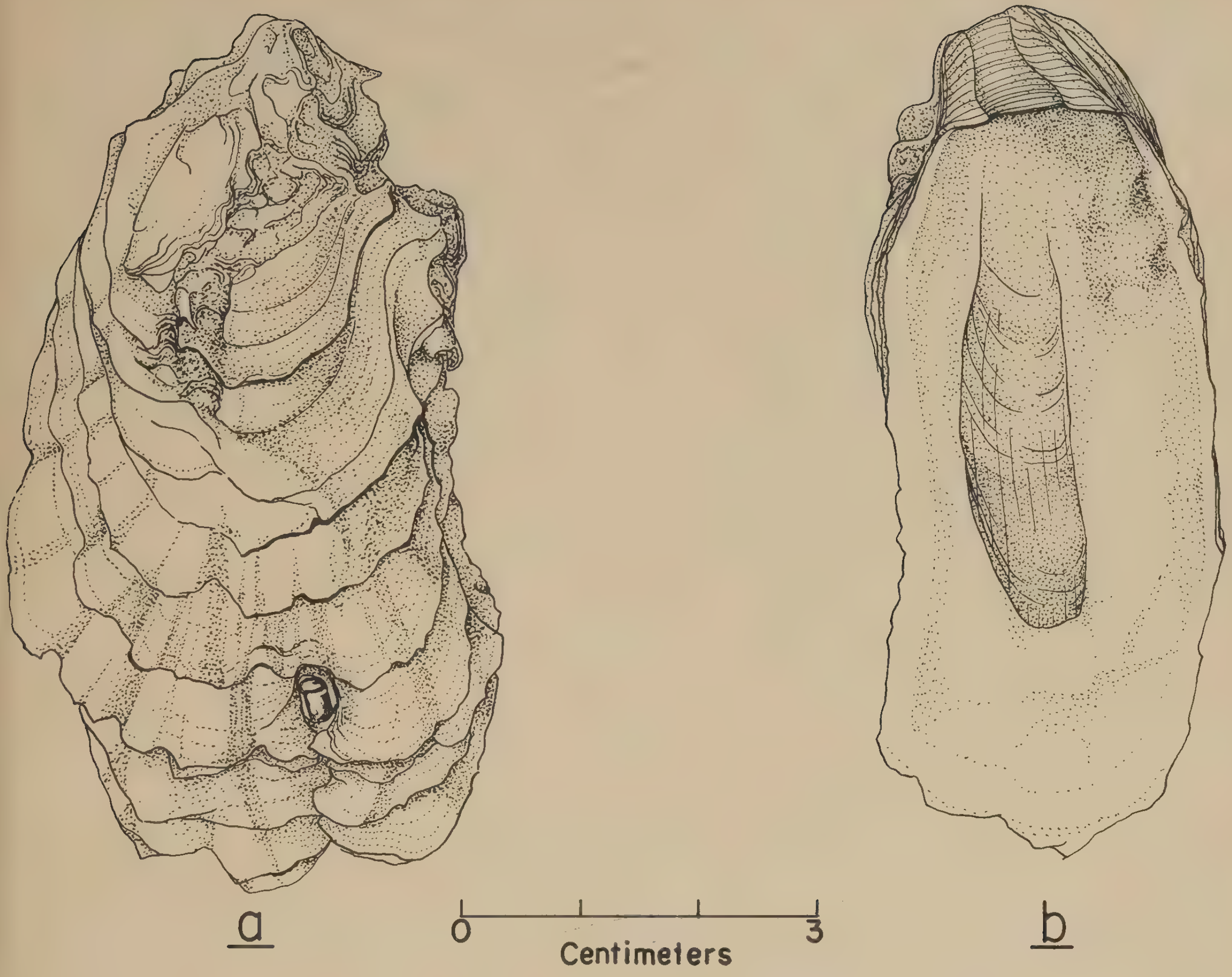

FIGURE 5.—*C. rhizophorae.* On mangrove roots, Florida Keys. Dimensions: height 7 cm., length 3.7 cm. (a) exterior; (b) interior of the shell.

most smooth, sometimes covered with tubular projections. The color of the right valve is cream buff with many radial chocolate bands; their arrangements greatly variable. Muscle scar, situated near the center or a little dorsally, is white, occasionally with olive-ochre spots. Margin of the mantle is dark violet; the tentacles are arranged in two rows; those of the outer row are of irregular size; the inner tentacles in a single row are slender.

The species, described from China by Gould, occurs in Ariake Bay and in the bays of Okayama Prefecture, Japan. It has established itself in Puget Sound.

Ostrea edulis Linné (fig. 9). This European flat oyster is the type species of the genus *Ostrea*. Shell round or oval; left valve larger and deeper, slightly bulging with 20 to 30 ribs and irregular concentric lamellae. Upper valve smaller, flat, without ribs, with numerous concentric lamellae. Beaks poorly developed. Ligament consists of three parts; the middle part is flat on the left valve and forms a projection on the right valve. Muscle scar is eccentrically located, unpigmented. Promyal chamber absent. Ostia and eggs relatively large. Hermaphroditic and incubatory mollusk, discharging eggs into the gill cavity. Small numbers of European flat oysters were introduced several years ago into the coastal waters of Maine in Boothbay Harbor where they survived and reproduced themselves. At present the population is too small to be used commercially. Recently the stock of European oysters in Maine waters was increased by planting seed raised from eggs fertilized and developed in the Bureau of Commercial Fisheries Biological Labora-

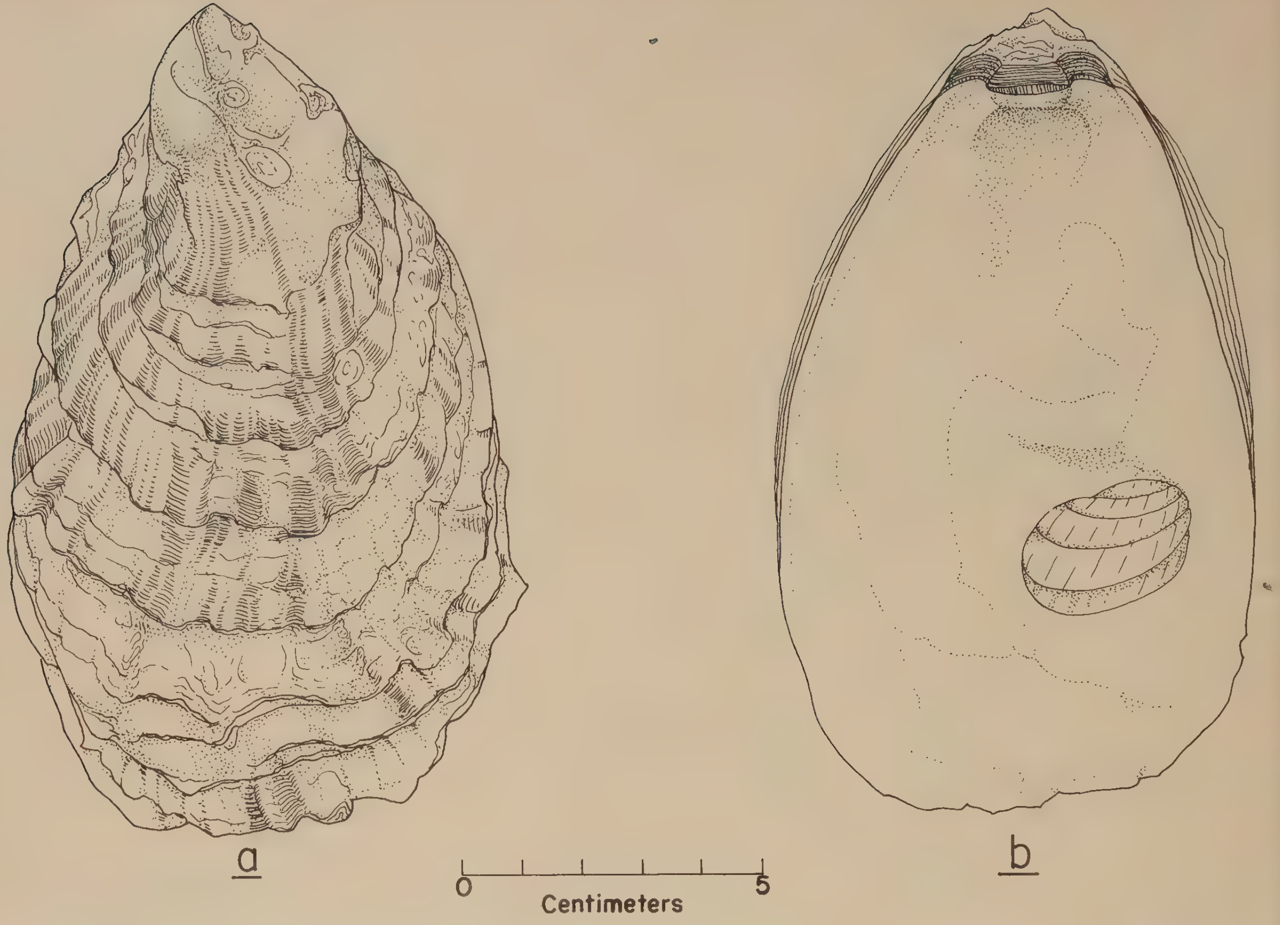

FIGURE 6.—*C. gigas*. Adult oyster grown in Willapa Harbor, Wash., from seed imported from Japan. Dimensions: height 14 cm., length 9 cm. (a) exterior of lower (left) valve; (b) interior of upper (right) valve.

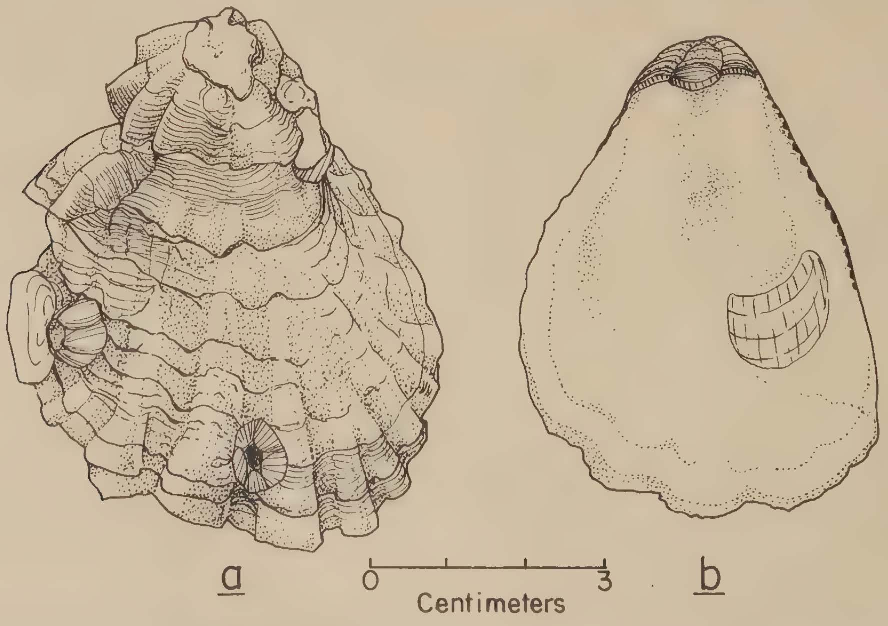

FIGURE 7.—*C. commercialis*. Kaneohe Bay, Oahu Island, Hawaii. Dimensions: height 7 cm., length 4.8 cm. (a) exterior of lower (left) valve; (b) interior of upper (right) valve.

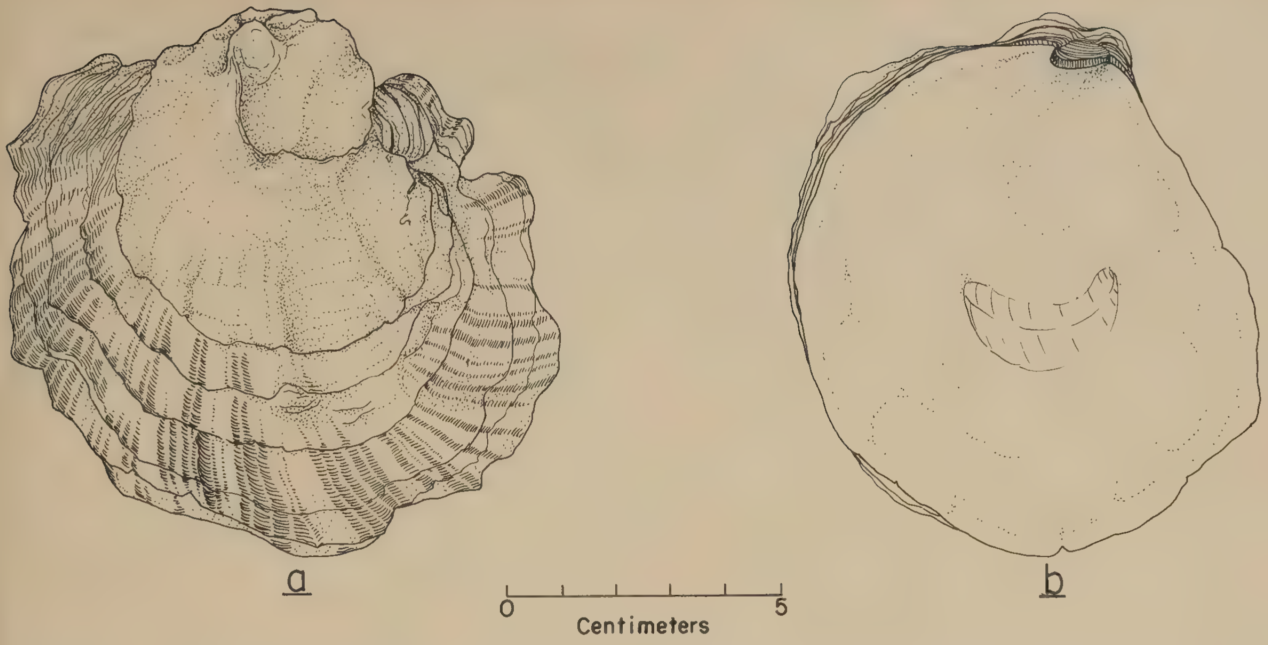

FIGURE 8.—*C. rivularis.* Puget Sound. Introduced from Japan with the seed of *C. gigas.* Dimensions: height 10.7 cm., length 10.5 cm. (a) exterior of lower (left) valve; (b) interior of upper (right) valve.

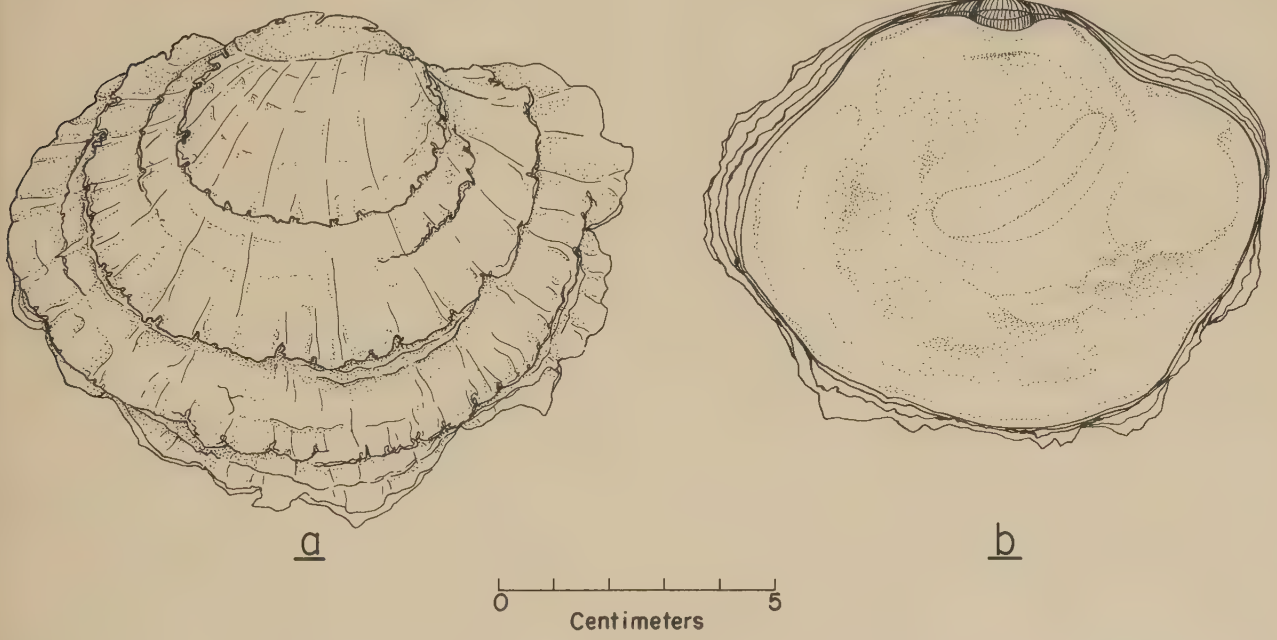

FIGURE 9.—*O. edulis,* European flat oyster introduced from Europe to Boothbay Harbor, Maine. Dimensions: height 9 cm., length 11 cm. (a) exterior of the left (lower) valve; (b) interior of the right (upper) valve.

tory at Milford, Conn. Prodissoconch shown in fig. 350 ch. XVI.

O. lurida Carpenter (fig. 10). Shells from 2 to 3 inches in height, with coarse concentric lines. Inner side of valves usually olive-green. Promyal chamber absent. Hermaphroditic, incubatory, eggs retained in the gill cavity until fully developed larvae are formed and discharged into the water. Inhabits the tidal waters of the Pacific Coast of North America from Alaska to Lower California. (Prodissoconch shown in fig. 351, ch. XVI.).

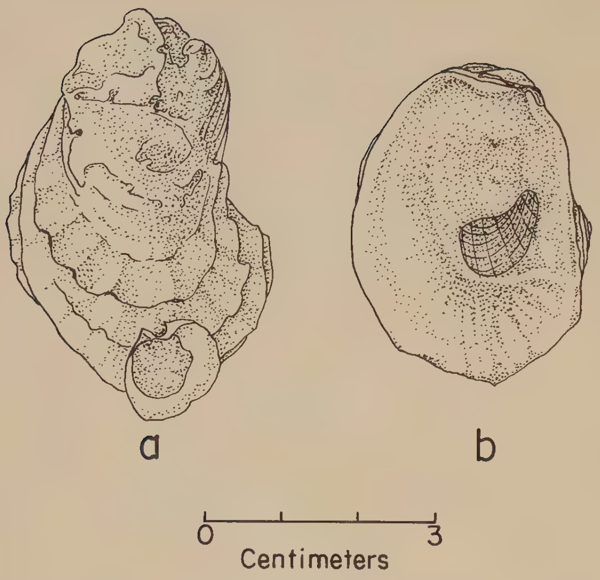

FIGURE 10.—*O. lurida* from Puget Sound. Left (a) and right (b) valves. Height 5.5 cm.; length 3 cm.

O. equestris Say, horse oyster, crested oyster (fig. 11). This small, noncommercial oyster of the South Atlantic states, Gulf of Mexico, and West Indies is often mistaken by laymen for young *C. virginica.* Its average size is about 2 inches, but occasionally specimens measuring up to 3½ inches in height are found. Say (1834), who described the species, lists the following identifying characters: Shell small, with transverse wrinkles, and more or less deeply and angularly folded longitudinally; ovate-triangular, tinted with violaceous; lateral margins near the hinge with from 6 to 12 denticulations of the superior valve; superior valve depressed but slightly folded; inferior valve convex, attached by a portion of its surface, the margins elevated, folds unequal, much more profound than those of the superior valve; hinge very narrow, and curved laterally and abruptly.

The shape of the oyster shell is very variable depending on crowding and type of substratum. The most significant combination of features by which *O. equestris* can be distinguished from small *C. virginica* and from *O. frons* are as follows: a rather high vertical and crenulated margin of the lower valve; off-center position of the adductor muscle scar; a dull greenish color of the interior surface; and the presence of a single row of denticles of the upper valve with the corresponding depression on the lower valve (Galtsoff and Merrill, 1962). The number of denticles and their size very variable. Ranson (1960) does not include *O. equestris* in the drawings of prodissoconchs reproduced in his publication. A rough sketch of the prodissoconch of *O. equestris*, not showing the details of hinge structure, is given by Menzel (1953).

This incubatory species frequently occurs in large numbers on commercial oyster beds in association with *C. virginica.* It thrives in waters of high salinity (35‰) but has been found in regions where salinity is about 20 to 25‰. The northernmost boundary of distribution established by Merrill is about half way between Delaware and Chesapeake Bays, (37°31′ N., 73°18′ W.) at the depth of 60 fathoms.

O. frons Linné (fig. 12). Small shells of 1 to 2 inches in height with radial plicated sculpture and sharply folded margins. Valves closely set with minute denticles almost around the entire circumference. Muscle scar near the hinge. Beaks slightly curved. Interior white, exterior usually purple-red. Frequently attached to branches or roots of mangrove trees by a series of hooked projections. Common in Florida, Louisiana, and the West Indies. Was found attached to navigation buoys off Port Royal, S.C., and off Miami Harbor, Fla. (personal communication of A. S. Merrill).

O. permollis Sowerby (fig. 13). This small oyster lives commensally, completely embedded in sponges with only the margins of the valves visible. The species is common along the west coast of Florida, north of Tarpon Springs, where it is constantly associated with the sponge *Stellata grubi* (fig. 14). Rarely exceeds 1.5 inches in size. Surface of valves soft and silky. Beaks twisted back into a strong spiral. Inner margins with numerous small denticles. Nonedible species. North Carolina to Florida and the West Indies.

Pycnodonte hyotis Linné. This species dredged from 300 feet of water off Palm Beach was described by McLean (1941) as *O. thomasi*, nova species, but identified by Ranson as *Pycnodonte hyotis.* It is characterized by the peculiar foamlike appearance of its shell structure, particularly at the margins. Shells circular, 3 to 4 inches in diameter.

Pycnodonte is frequently found on navigation buoys off Key West, Fla., and near the entrance to Miami Harbor (personal communication of A. S. Merrill).

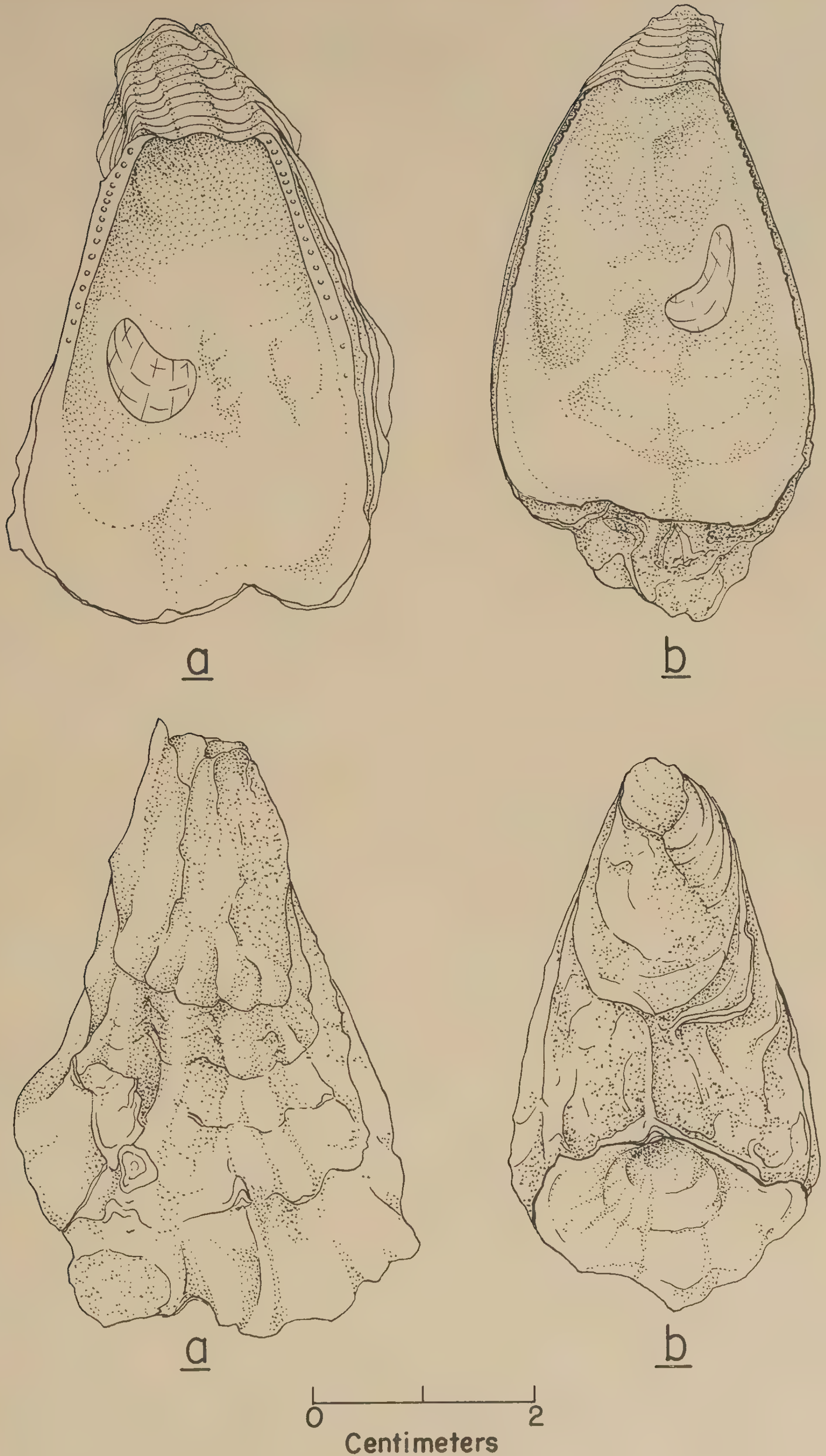
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FIGURE 11.—*O. equestris*. Left (a) and right (b) valves. 5 cm. in height.

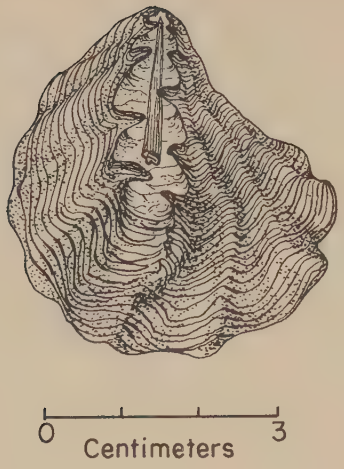

Figure 12.—*O. frons.* Key Biscayne, Florida. Natural size.

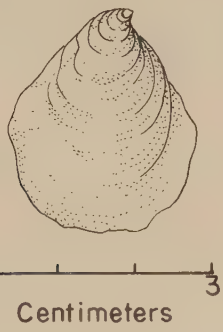

Figure 13.—*O. permollis.* West of Andote Light, Tarpon Springs, Fla., at 6 fathoms. Dimensions: height 3.5 cm., length 3 cm.

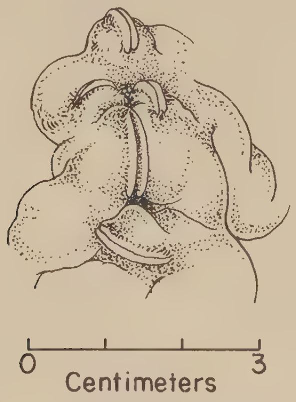

Figure 14.—*O. permollis* embedded in sponge *Stellata grubi.*

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CHAPTER II

MORPHOLOGY AND STRUCTURE OF SHELL

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APPEARANCE AND PRINCIPAL AXES

The body of the oyster is covered with two calcareous valves joined together by a resilient ligament along the narrow hinge line. The valves are slightly asymmetrical. The left one is larger and deeper than the right one, which acts as a lid. Under normal conditions the oyster rests on the left valve or is cemented by its left valve to the substratum. The difference between the right (flat) and left (cuplike) valve is to a certain degree common to all the species of oysters which have been sufficiently studied. Orton's (1937) statement with reference to *Ostrea edulis* that: "In life the flat or right valve usually rests on the sea bottom and is often referred to as the lower one" is an obvious oversight.

In *C. virginica* the left valve is almost always thicker and heavier than the right one. When oysters of this species are dumped from the deck of a boat and fall through water they come to rest on their left valves. I observed this many times while planting either small oysters not greater than 2 inches in height, or marketable adults of 5 to 6 inches. In the genus *Ostrea* the difference between the two valves is not great, it is greater in the genus *Crassostrea*, and extremely pronounced in the oyster of uncertain systematic position from Australia which Saville-Kent (1893) has called "*Ostrea mordax* var. *cornucopiaeformis*." [2]

The oyster is a nearly bilaterally symmetrical mollusk with the plane of symmetry passing between the two valves parallel to their surfaces. In orienting any bivalve it is customary to hold it vertically with the narrow side uppermost (fig. 15). The narrow end or apex of the shell is called the umbo (plural, umbos or umbones) or beak. A band of horny and elastic material, the ligament (fig. 16) joins the valves at the hinge on which they turn in opening or closing the shell.

In many bivalves the hinge carries a series of interlocking teeth, but these structures are absent in the family Ostreidae. The hinge consists of the following parts: a projecting massive structure within the right valve, the buttress, according to Stenzel's terminology, supports the midportion of the ligament and fits the depression on the left valve. The tract made by the buttress during the growth of the shell along the midportion of the ligamental area is the resilifer. On the left valve the resilifer is the tract left on the depression. The central part of the ligament is called resilium.

The pointed end of the valve or the beak represents the oldest part of a shell. In old individuals it reaches considerable size (fig. 17). The beaks are usually curved and directed toward the posterior end of the mollusk although in some specimens they may point toward the anterior. In the majority of bivalves other than oysters the beaks usually point forward. The direction and degree of curvature of the beaks of oysters as well as their relative proportions vary greatly as can be seen in figure 18, which represents different shapes found in old shells of *C. virginica*. Very narrow, straight, or slightly curved beaks of the kind shown in figure 18–1 are usually formed in oysters which grow on soft, muddy bottoms. Extreme development of this type can be seen in the narrow and slender oysters growing under overcrowded conditions on reefs (fig. 19). Other forms of beaks (fig. 18, 2–4) cannot be associated with any particular environment. In fully

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[2] I am indebted to H. B. Stenzel for calling my attention to this species and for several suggestions regarding the morphological terminology used in this chapter.

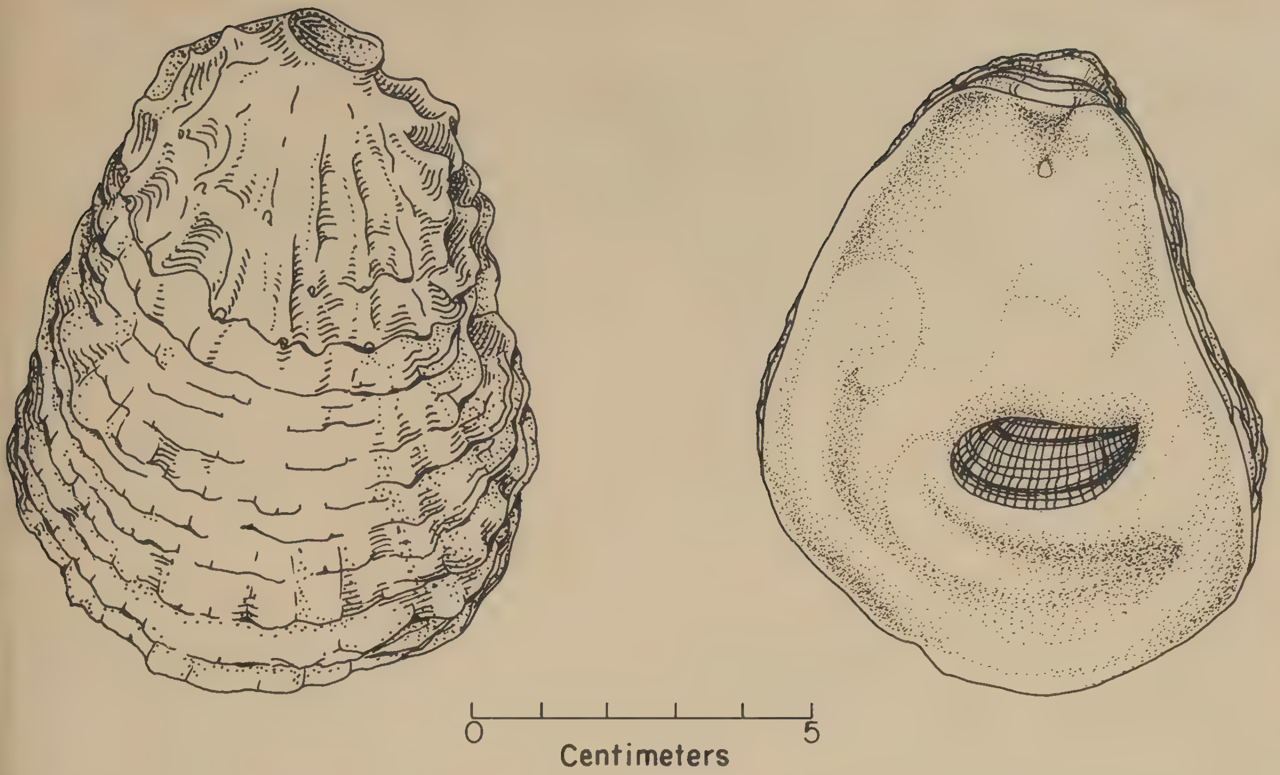

FIGURE 15.—Blue Point oyster (*C. virginica*) from Great South Bay, Long Island, N.Y. The size of this 5-year-old oyster is about 10 x 6.6 cm. (4 x 3 inches). The shell is strong and rounded; its surface is moderately sculptured. Left—outside surface of left valve. Right—inner surface of right valve. Small encircled area under the hinge on the inner surface of right valve is an imprint of Quenstedt's muscle.

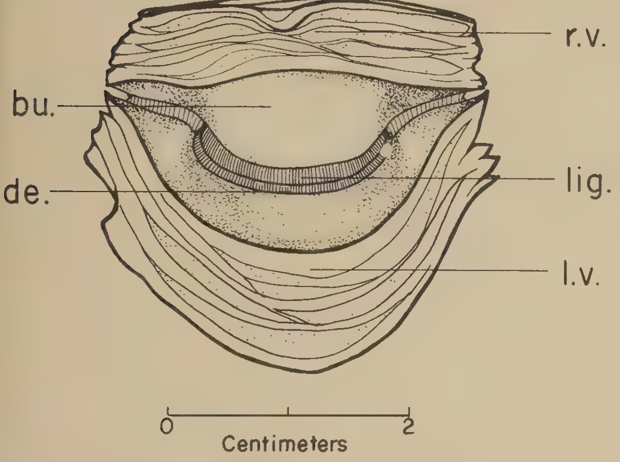

FIGURE 16.—Cross section below the hinge of an adult *C. virginica*. Left valve at bottom, right valve at top of the drawing. The buttress of the right valve fits the depression on the left valve. The two valves are connected by a ligament (narrow band indicated by vertical striations) which consists of a central part (resilium) and two outer portions. Slightly magnified. r.v.—right valve; bu.—buttress; de.—depression or furrow on left valve (l.v.); lig.—ligament.

grown *C. virginica* the pointed end of the upper (flat) valve is always shorter than that of its opposite member (fig. 17). The angle between the two beaks determines the greatest extent to which the valves can open for feeding or respiration and is, therefore, of significance to the oyster.

If the oyster shell is oriented in such a way that both of its valves are visible and the beaks point up and toward the observer, the flat valve with a shorter, convex resilifer is the right one and the cuplike valve with the longer concave resilifer is the left one. The dorsal margin of the oyster is the beak or hinge side, the ventral margin the opposite. If viewed from the right (flat) valve with the hinge end pointing away from the observer the anterior end of the oyster is at the right side of the valves and the posterior is at the left.

The posterior and anterior parts of the oyster shell may also be identified by the position of the muscle impression, an oval-shaped and highly pigmented area marking the attachment of the adductor muscle on the inner side of each valve.

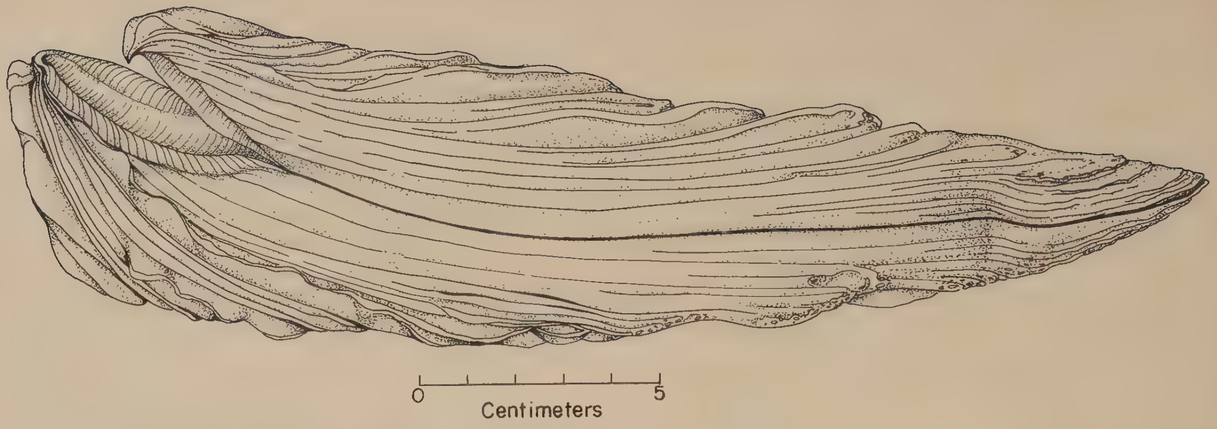

FIGURE 17.—Side view of a very old and large *C. virginica* from Stony Creek, Conn. Notice the curvature of the beak, the depressed resilifer on the lower valve and the protruding resilifer on the upper one. The angle between the beaks determines the maximum movement of the upper valve. Dimensions: height—25.5 cm. (10 inches) and width—6.4 cm. (2.5 inches).

The muscle impression is asymmetrically located closer to the posterior end of the valve. This area of the attachment of the adductor muscle has been called the "muscle scar." Some malacologists prefer to use the expression "muscle impression" or "area of attachment" (Stenzel, personal communication) because the word "scar" usually means the mark left by healing of an injury. The proposed change in terminology does not seem to be desirable because the name "muscle scar" has been so well established in scientific and popular writings that its abandonment may cause confusion.

The three principal dimensions of bivalves, including oysters, are measured in the following manner (fig. 20): height is the distance between the umbo and the ventral valve margin; length is the maximum distance between the anterior and posterior margin measured parallel with the hinge axis; and width is the greatest distance between the outsides of the closed valves measured at right angles to the place of shell commissure.

In many popular and trade publications on shellfish the word "length" is used instead of "height", and the word "width" is employed to designate the length of the oysters. To avoid confusion the scientific rather than popular terminology is used throughout the text of this book.

The shape of oyster shells and their proportions are highly variable and, therefore, are, in some cases, of little use for the identification of species. The variability is particularly great in the species of edible oysters (*C. virginica*, *C. gigas*, *C. angulata*, and *C. rhizophorae*) that have a wide range of distribution, thrive on various types of bottom, and are tolerant to changes in salinity and turbidity of water. Certain general relationships between the shape of the oyster shell and the environment are, however, apparent in *C. virginica*. Oysters growing singly on firm bottom have a tendency to develop round shells ornamented with radial ridges and foliated processes (figs. 4, 15). Specimens living on soft, muddy bottoms or those which form clusters and reefs are, as a rule, long, slender, and sparsely ornamented (figs. 19, 21).

The thickness and strength of the valves of *C. virginica* are highly variable. Shells of oysters grown under unfavorable conditions are often thin and fragile (Galtsoff, Chipman, Engle, and Calderwood, 1947). Likewise, so-called "coon" oysters from overcrowded reefs in the Carolinas and Georgia are, as a rule, narrow and have light shells (fig. 19). Heavy and strong shells are not typical for any particular latitude. They can be found on hard, natural bottoms throughout the entire range of distribution of *C. virginica*. I have in my collection shells from Prince Edward Island, Cape Cod, Delaware Bay, Louisiana, and Texas which in shape and strength of valves are indistinguishable from one another. Sometimes the growth of shells in length (in anteroposterior direction) equals or exceeds the growth in height. Such specimens, one from Texas and one from the waters of Naushon Island off the Massachusetts coast, were found in sticky mud. As can be seen

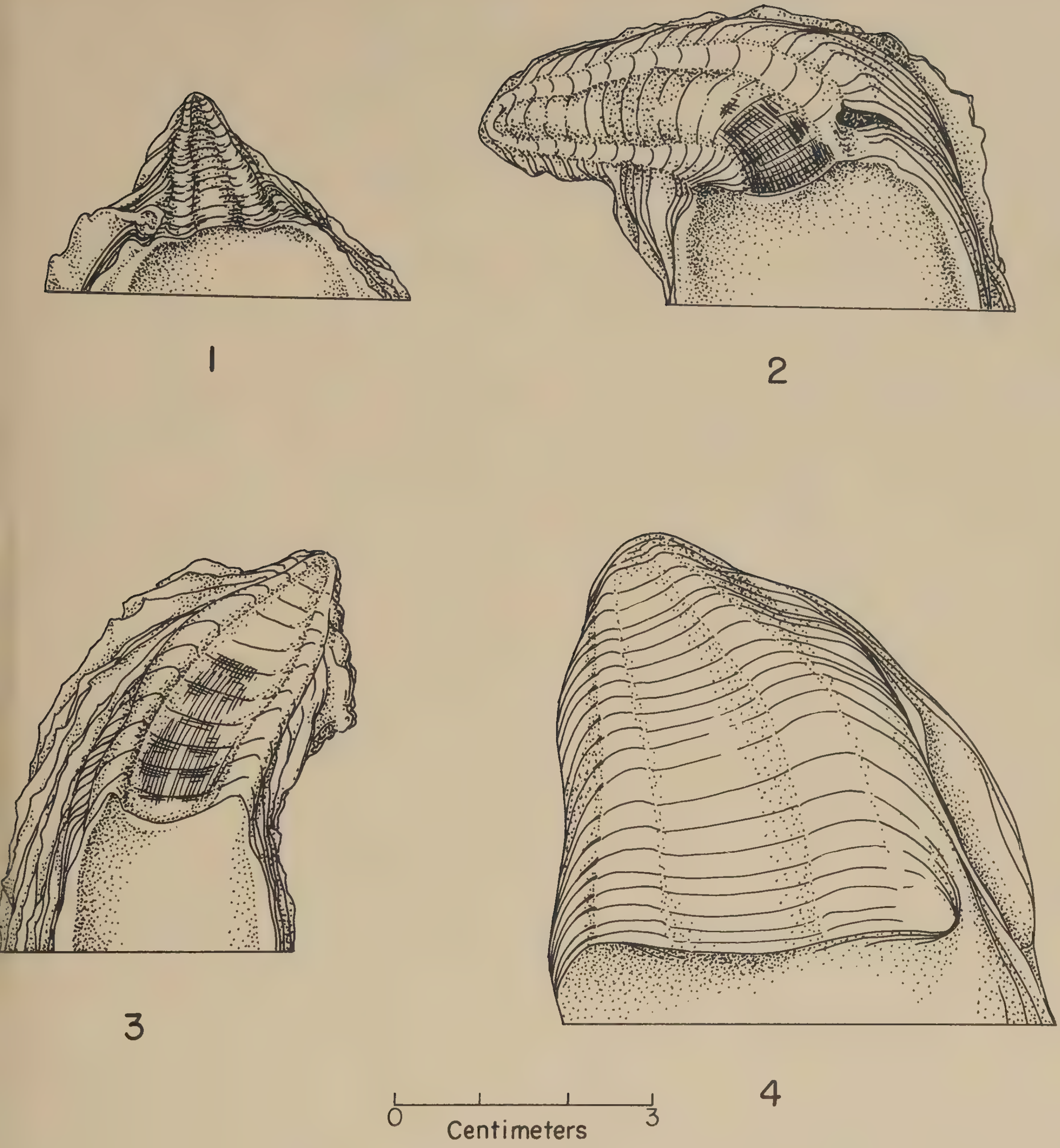

FIGURE 18.—Four shapes of beaks on left valves of old oysters, *C. virginica*. 1—narrow, short and almost straight; 2—strongly curved to the posterior; 3—of medium width, pointed forward; 4—very broad and slightly curved to the posterior.

rom figure 22, the shells are almost identical in hape and size.

Oysters are frequently marketed under specific rands or trade names such as Blue Points (fig. 5), Cotuits, Chincoteagues, and others which imply the existence of local varieties different in size and shape of shells. There is no evidence, however, to substantiate this claim. So-called "Blue Points" characterized by round shape, strong shell, and medium size may be found,

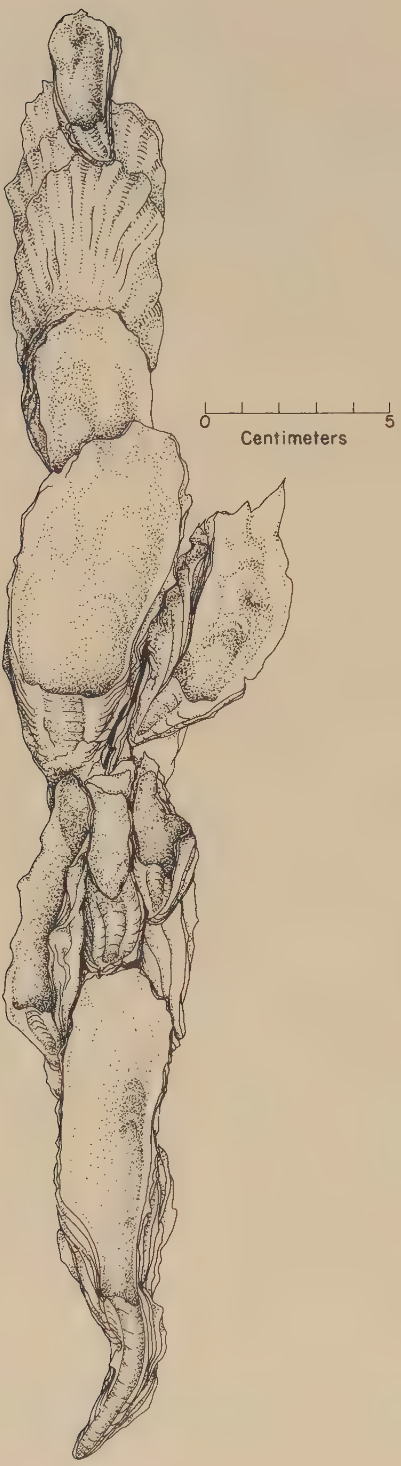

FIGURE 19.—Several generations of oysters, *C. virginica*, growing vertically on muddy bottom of Altamaha Sound, Ga. Notice the very long and narrow beak of the lowermost shell.

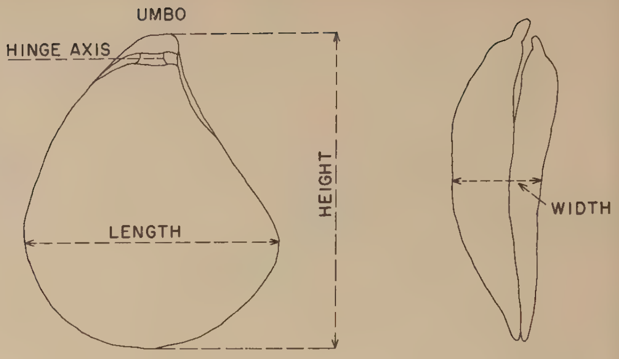

FIGURE 20.—Diagram showing the correct method of measuring the height, length, and width of oyster shells.

for instance, in any part of the coast where oysters grow singly on hard bottom and are not crowded. As a matter of fact, in past years "Blue Points" sold in retail stores actually were taken from the Chesapeake Bay and North Carolina. This is also true for "Cotuits" and other popular brands.

That the shape of oysters cannot be associated with any particular geographical location is best shown by the fact that all the kinds represented in trade, including long and narrow "coon" oysters which are regarded as being typical for the tidal areas of the South Atlantic States, are found in various bays and estuaries of Cape Cod, Mass. The only shell character that appears to be associated with the geographical distribution of the species is the pigmentation of the interior surfaces of the valves. In North Atlantic oysters the inner surface is unpigmented or very lightly pigmented (outside of the place of attachment of the adductor muscle), while in South Atlantic and Gulf oysters the dark brown or reddish pigmentaton of the valves is more pronounced.

DIMENSIONS

Oysters (*C. virginica*) of marketable size usually measure from 10 to 15 cm. (4 to 6 inches) in height; depending on the place of origin an oyster of this size may be 3, 4, or 5 years old.

As a rule, oysters do not stop growing after reaching certain proportions but continue to increase in all directions and, consequently, may attain considerable size. Such old and very large oysters are usually found on grounds undisturbed by commercial fishing. The largest oyster in my collection was found in the vicinity of Boothbay Harbor, Maine. Its dimensions were as follows:

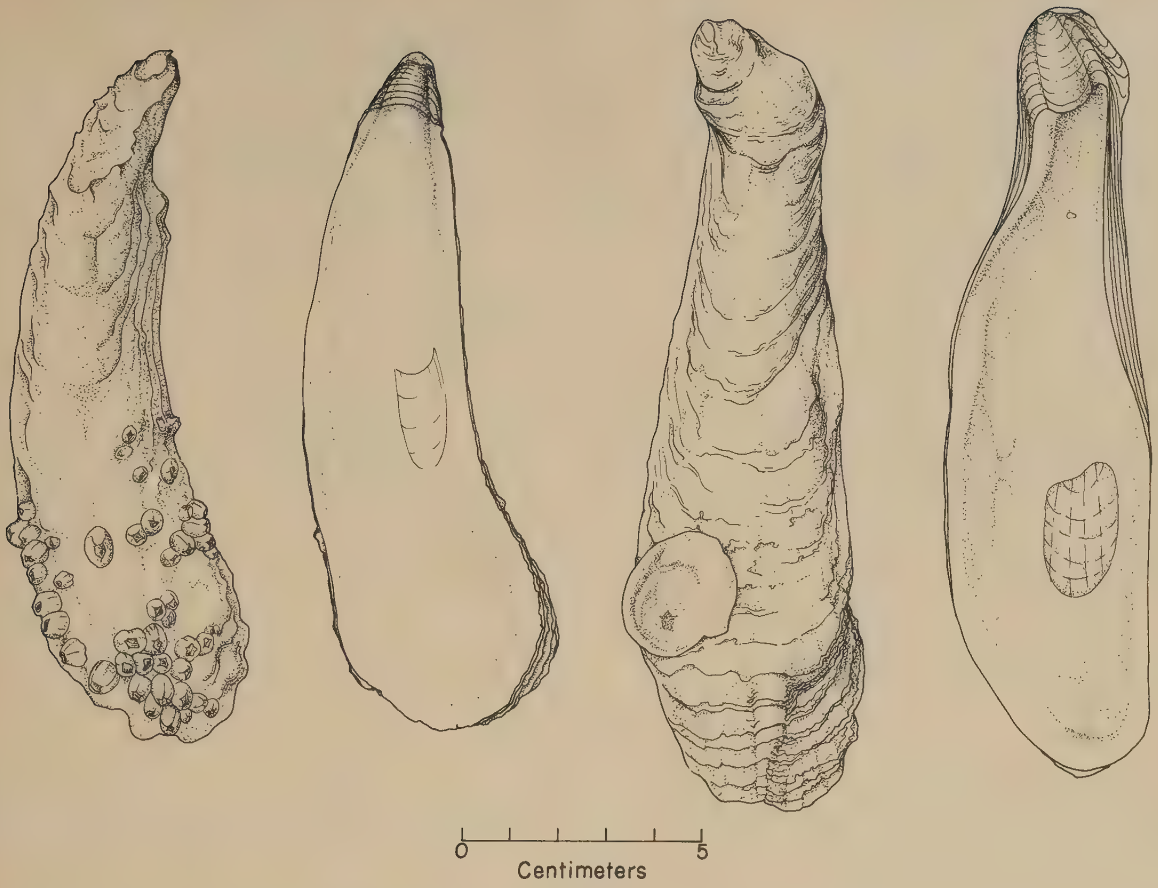

Figure 21.—Shells of *C. gigas* (left) and *C. virginica* (right) grown on soft, muddy bottom. Note the remarkable similarity in the shape, size, and sculpture of the two species of oysters. The *C. gigas* was obtained from the northern part of Puget Sound and the *C. virginica* from Georgia. The shells of the two species can be distinguished by the absence of pigmentation of the muscle impression in *C. gigas* and by its lighter shell material.

height—20.6 cm. (8.1 inches); height of left and right beak—5.5 cm. (2.1 inches) and 4.5 cm. (1.75 inches) respectively; length of shell—9.7 cm. (3.8 inches); maximum width (near the hinge)—6.5 cm. (2.6 inches). The total weight was 1,230 g., the shell weighing 1,175 g., the meat 35.8 g., and the balance of 19.2 g., representing the weight of sea water retained between the valves. Apparently the largest oyster recorded in American literature is the giant specimen from the Damariscotta River, Maine, reproduced in natural size by Ingersoll (1881, pl. 30, p. 32). This shell is 35.5 cm. (14.3 inches) in height and 11 cm. (about 4.4 inches) in length.

SHAPE OF SHELLS

The shells of many gastropods and bivalves are spiral structures in which the convolutions of the successive whorls follow a definite pattern. The spiral plan is frequently accentuated by ridges, furrows, spines and nodules, or by pigmented spots which repeat themselves with remarkable regularity. A spiral structure is not restricted to mollusk shells. As a matter of fact, it is very common throughout the animal and plant kingdom as well as in architecture and art. Examples of a great variety of spirally built organisms and structures are given in the beautifully illustrated books entitled "Spirals in nature and art" and "Curves of life" (Cook 1903, 1914). As the title of the second book implies, Cook is inclined to attach some profound significance to the kind of curves found in animal and plant forms. This view, inherited from the philosophers of the 18th and 19th centuries, considers the spiral organic structures as a manifestation of life itself. The influence of this philosophy persisted among some scientists until the thirties of the present century. It can be found, for instance, as late as 1930 in the writings of a French physiologist, Latrigue (1930)

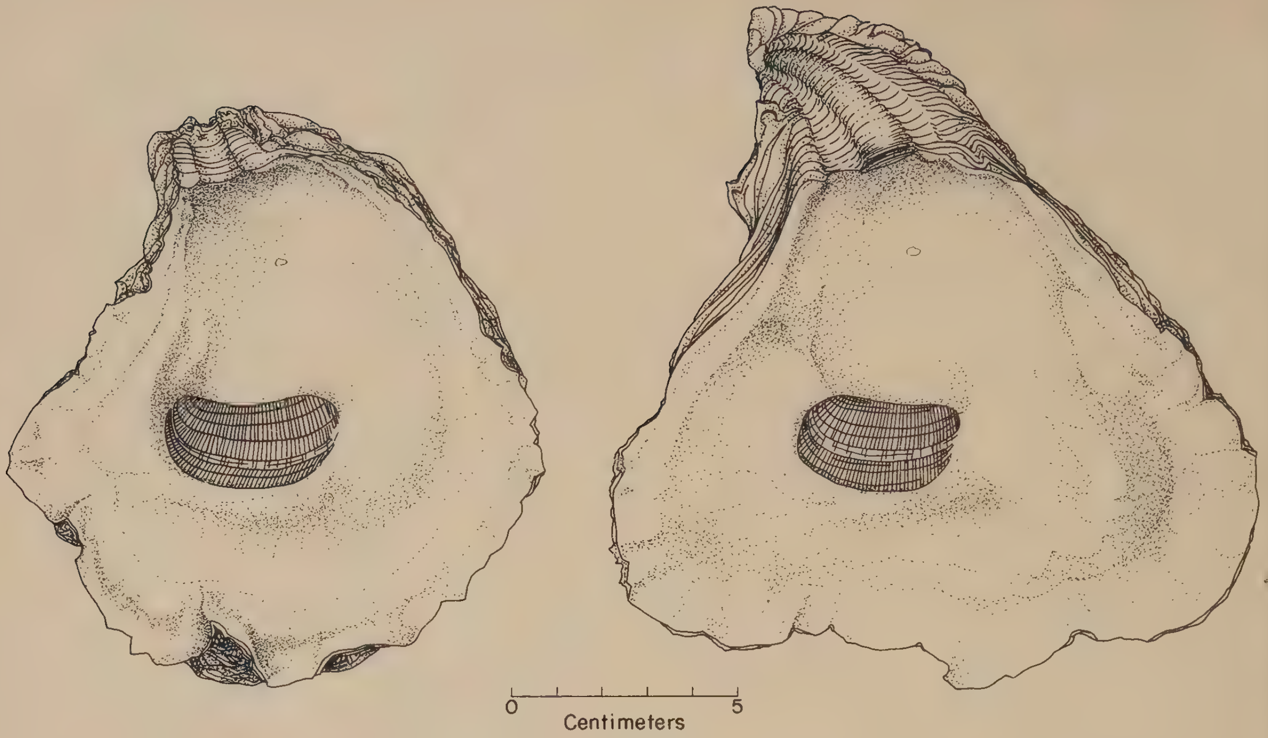

FIGURE 22.—Two left shells of *C. virginica* grown on sticky mud. On the left side is the oyster from Karankawa Reef in Matagorda Bay, Tex.; on the right is the oyster from Hadley Harbor, Naushon Island, near Woods Hole, Mass. The dimensions of the Texas oyster are 13 by 11.5 cm. (5.1 by 4.5 inches) and for the Hadley Harbor oyster 15.5 by 14.5 cm. (6.1 by 5.7 inches).

who in the book, "Biodynamique générale," attributes mysterious and not well-defined meaning to the "stereodynamics of vital vortex." These speculations contributed nothing to the understanding of the processes which underlie the formation of shells and other organic structures.

In the earlier days of science the geometric regularity of shells, particularly that of gastropods, had been a favored object for mathematical studies. Properties of curves represented by the contours of shells, as well as those seen in horns, in flower petals, in the patterns of distribution of branches of trees, and in similar objects, were carefully analyzed. An excellent review of this chapter of the history of science is given in a well-known book "On growth and form" (Thompson, 1942) in which the reader interested in mathematics and its application to the analysis of organic forms will find many stimulating ideas.

Among the array of curves known in mathematics, the kind most frequently encountered in the shells of mollusks is the logarithmic or equiangular spiral (fig. 23). The latter name refers to one of its fundamental characteristics, described by Descartes, namely, that the angle between tangent PG (fig. 23) and radius vector OP is constant. Another property of this curve which may be of interest to biologists is the fact that distances along the curve intercepted by any radius vector are proportional to the length of these radii. D'Arcy Thompson showed that it is possible to apply the mathematical characteristics of curves to the interpretation of the growth of those shells which follow the pattern of a logarithmic spiral. According to his point of view, growth along the spiral contour is considered as a force acting at any point P (fig. 23) which may be resolved into two components PF and PK acting in directions perpendicular to each other. If the rates of growth do not change, the angle the resultant force, i.e., the tangent PG, makes with the radius vector remains constant. This is the fundamental property of the "equiangular" (logarithmic) spiral. The idea forms the basis of Huxley's (1932) hypothesis of the interaction of two differential growth ratios in the bivalve shells and also underlies Owen's (1953) concept of the role of the growth components determining the shape of the valves.

Another important characteristic of the growth of bivalves pointed out by Thompson is that

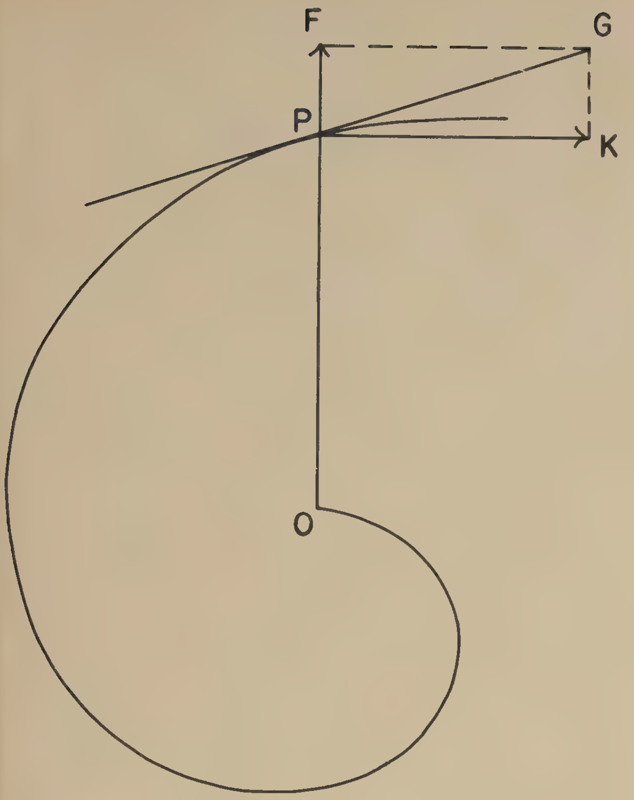

Figure 23.—Logarithmic or equiangular spiral. Explanation in text.

increase in size is not accompanied by any change in shape of the shell; the proportions of the latter remain constant, and the shell increases only in size (gnomonic growth). This general rule holds true for many free-moving gastropods and bivalves. It is not, however, applicable to sessile forms like oysters, in which the shape of the shell changes somewhat with size, particularly at the early stages of growth, and is greatly modified by contact with the substratum upon which the mollusks rest. The plasticity and variability of attached forms are probably associated with their inability to escape the effects of proximate environment.

The contour of oyster shell may be either circular (young *C. virginica*, *O. edulis*) or elongated and irregular. Spiral curvature may be noticed, however, on a cross section of the lower (concave) valve cut along its height perpendicular to the hinge. The curve can be reproduced by covering the cut surface with ink or paint and stamping it on paper. The upper valve is either flat or convex.

The curvature of bivalve shells is sometimes called conchoid. The term may be found in general and popular books dealing with bivalve shells, but the author who introduced it in scientific literature could not be traced. The Greek word "conchoid", derived from "conch"—shell and "eidos"—resembling or similar to, implies the similarity of the curve to the contour of a molluscan shell.

The curve is symmetric with respect to the 90° polar axis (fig. 24). It consists of two branches, one on each side of the fixed horizontal line CD to which the branches approach asymptotically as the curve extends to infinity. The curve, known as conchoid of Nicomedes, is constructed by drawing a line through the series of points P and P_1 which can be found in the following way: from the pole O draw a line OP which intersects the fixed line CD at any point Q. Lay off segments $QP=QP_1=b$ along the radius vector OP. Repeat the process along the radii originating from the pole O and draw the two branches of the curve by joining the points. The curve has three distinct forms depending on whether "a" (a distance OQ from the pole to the point of intersection of the polar axis with the fixed line CD) is greater, equal to or less than b. The formula of the curve if $b<a$, is $r=a \sec \theta \pm b$, where r is the locus of the equation and $\sec \theta$ is secant of the vectorial angle θ.

Sporn (1926) made a detailed mathematical analysis of the conchoid curve and considered that the curvatures of bivalve shells conform to this geometrical type. Lison (1942) rejected this conclusion as not supported by observations and experimental evidence. He quite correctly stated that Sporn's work deals exclusively with abstract mathematical analyses of curves which in reality are not those found in molluscan shells. If one cuts a bivalve shell at any angle to the plane of closure of the valves, one obtains the curved lines of the two valves (fig. 25) which only remotely resemble the conchoid of Nicomedes and touch

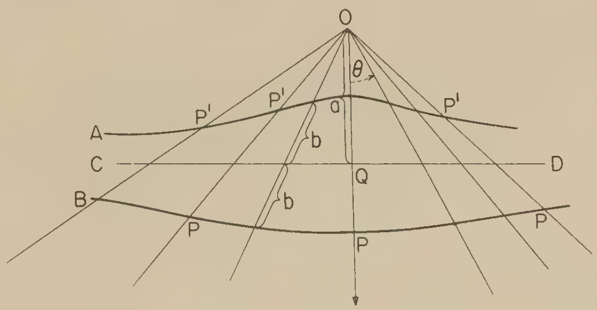

Figure 24.—Construction of the conchoid curve of Nicomedes. Explanation in text.

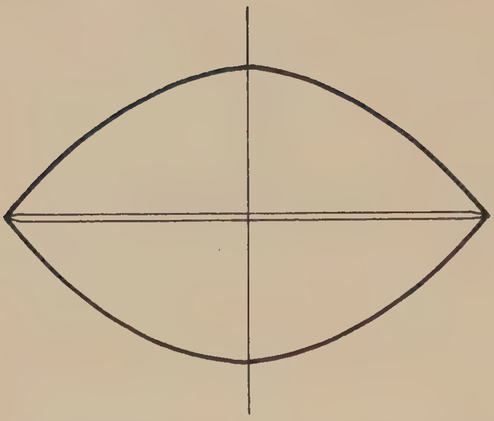

Figure 25.—Cross section of two valves of *Cardium*. The similarity with the conchoid in figure 24 is superficial.

each other at the ends. The two branches of conchoid (C and D) join together only in infinity (fig. 24).

Lison pointed out that the shape of the shell may be considered as a whole series (ensemble) of arches, the curvatures of which are described by logarithmic spirals of the same parameter which have a common origin at the umbo. The latter is their common pole. The arches terminate at the edge of the valve. The contour of the valve edge, frequently called the "generating curve", is usually confined to one plane parallel to the plane of opening and closing of the shell.

Among many spirals that can be drawn on the surface of a shell only one is completely confined to a single plane. This spiral was called by Lison the "directive spiral"; its plane is the "directive plane" of the shell. All other spirals which can be easily noticed on the shell surface as ridges, furrows, or as pigmented bands deviate to the right or left depending on which side of the directive plane they are located (fig. 26).

By mathematical analysis of the curved surfaces of various bivalve species Lison arrived at the general equation[3] of a valve. He observed that by itself such an equation may not be helpful to biologists unless it can be used for comparing the shape of the individuals of the same species or in making comparison between the different species. Lison stated that in practice it is not necessary to make the involved mathematical computations. It is sufficient to compare certain "natural" characteristics of shells, namely, the directive plane described above, the plane of closure of valves (or commissure plane), and the angle of

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[3] General equation of a valve as given by Lison (1939) is as follows: $\sigma = \sigma_0 px$; $\omega = \omega_0 + \alpha$; $z = z_0 e^{pa}$ in which p is a constant and σ_0, ω_0, and z_0 are the functions which express on cylindrical coordinates the form of the free edge of the valve when the directive plane is located within the xy and the origin of the coordinates is at the umbo. (Translation by Paul S. Galtsoff.)

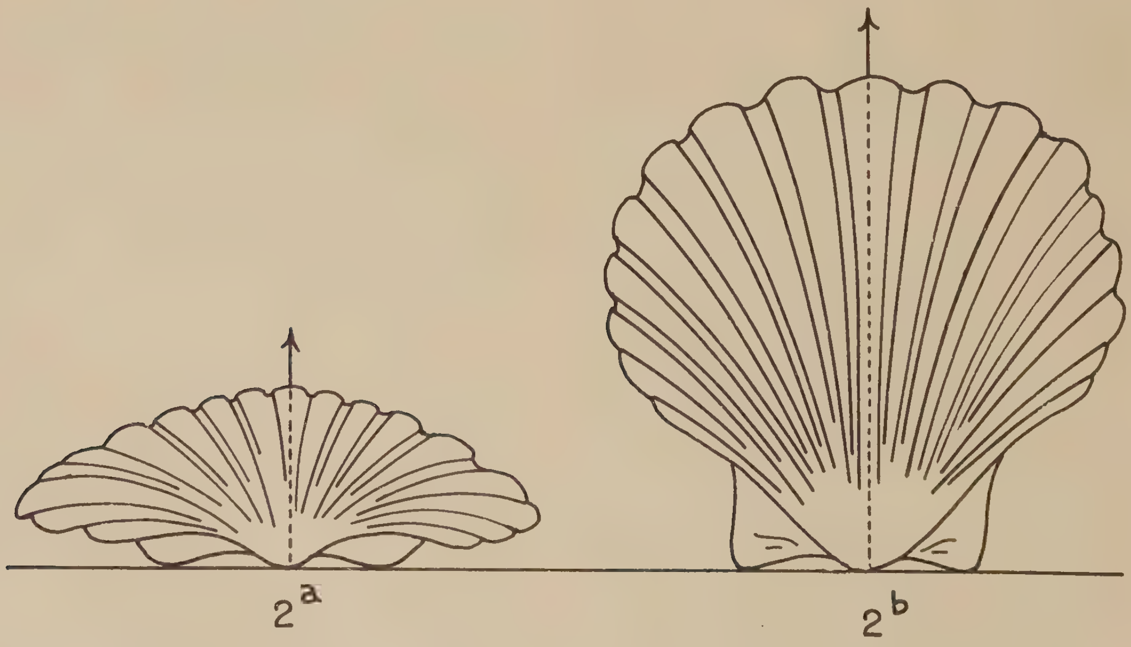

Figure 26.—Directive plane of scallop shell, *Pecten*, viewed from hinge end 2a, and from the broad side 2b. The arrows indicate the directive plane. (After Lison, 1939.)

incidence. The plane of closure of the valves originates at the umbo and passes between the edges of the two opposing valves when they are closed and touching each other. The angle of incidence, as defined by Lison, is the angle between the plane of closure and the directive plane. In round and symmetrical shells of scallops, pearl oysters, and other bivalves the directive plane is perpendicular to the plane of closure and the angle of incidence is 90° (fig. 26). In the shells of *Cardium orbita,* the directive plane forms an acute angle of 81° and is much smaller in elongated shells such as *Fimbria fimbriata* and *Trapezium oblongum.* The comparison between the shells can easily be made by recording the contours at the free margins of the valves and determining the angle of incidence.

To determine the shape of logarithmic spiral of the valve the shell may be sawed along the directive plane (fig. 27) and the section oriented with the umbo O at lower left. If S_1 and S_2 are respective lengths of the two radii the value of parameter p can be computed by using the fundamental equation of logarithmic spiral,

$$P = \frac{\log . S_1 - \log . S_2}{\omega}$$

(logarithms in this equation are natural, to base e).

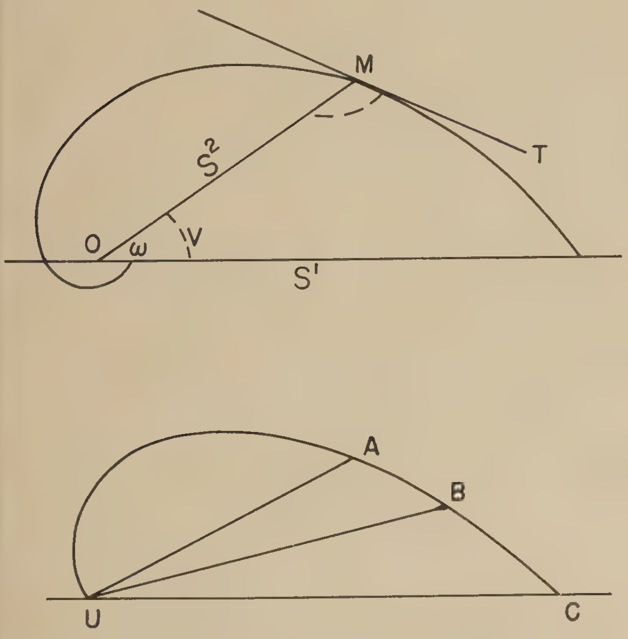

FIGURE 27.—Valve of a shell sawed along the directive axis describes a plane logarithmic spiral. According to Lison (1942). OM—radius vector; T—tangent; O—umbo; V—angle between the two radii.

In résumé, Lison attempted to prove that the form of the shell in which the generating curve is confined to one plane is determined by three conditions: (1) the angle of the directive spiral, (2) the angle of incidence, and (3) the outline of the generating curve.

Further attention to the problem of the shape and formation of the bivalve shell was given by Owen (1953). In general he accepted Lison's conclusions and stated that "the form of the valves should be considered with reference to: (a) the outline of the generative curve, (b) the spiral angle of the normal axis, and (c) the form (i.e., plani-spiral or turbinate-spiral) of the normal axis." The normal axis is considered by Owen with reference to: (1) the umbo, (2) the margin of the mantle edge, and (3) the point at which the greatest transverse diameter of the shell intersects the surface of the valves. Thus, it can be seen from this statement that Owen's "normal axis" does not coincide with Lison's directive plane except in bilaterally symmetrical valves (fig. 28). According to Owen's view, the direction of growth at any region of the valve margin is the result of the combined effect of three different components: (a) a radial component radiating from the umbo and acting in the plane of the generating curve, (b) a transverse component acting at right angles to the plane of the generating curve, and (c) a tangential component acting in the plane of the generating curve and tangentially to it. The turbinate-spiral form of some bivalve shells is due to the presence of the tangential component which in plani-spiral shells may be absent or inconspicuous. Likewise, the transverse component may be greatly reduced or even absent in the valve. Thus, from this point of view the great variety of shell forms may be explained as an interaction of the three components (fig. 29). Owen's point of

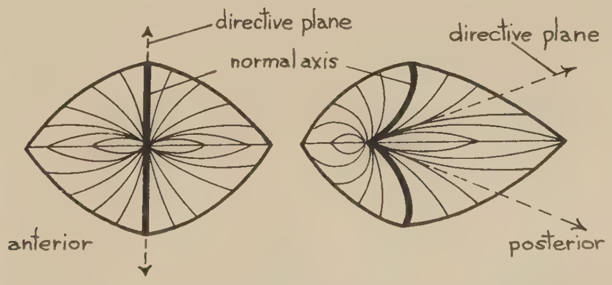

FIGURE 28.—Comparison of directive plane of Lison with normal axis (Owen). A—shell not affected by tangential component; B—shell affected by tangential component.

view is basically similar to Huxley's hypothesis (1932) of differential length growth and width growth of molluscan shells. Owen correctly points out an error in Lison's interpretations that the lines of equal potential activities involved in the secreting of shell material at the edges of the valves are parallel to each other. This is obviously not the case since all lines of growth of the lamellibranch shell radiate from a common node of minimum growth near the umbo. For this reason the comparison of bivalves can be more conveniently made by using radial coordinates as has been shown by Yonge (1952a, 1952b).

The mathematical properties of shell surfaces are of interest to the biologist because they may provide clues to understanding the quantitative aspects of the processes of shell formation. It can be a priori accepted that any organism grows in an orderly fashion following a definite pattern. The origin of this pattern and the nature of the forces responsible for laying out structural materials in accordance with the predetermined plan are not known. The pattern of shell structure is determined by the activities at the edge of the shell-forming organ, the mantle. At the present state of our knowledge it is impossible to associate various geometrical terms which describe the shape of the shell with concrete physiological processes and to visualize the morphogenetic and biochemical mechanisms involved in the formation of definite sculptural and color patterns. The solution of this problem will be found by experimental and biochemical studies which may supply biological meanings to abstract mathematical concepts and equations. Experimental study of the morphogenesis of shells offers splendid opportunities for this type of research.

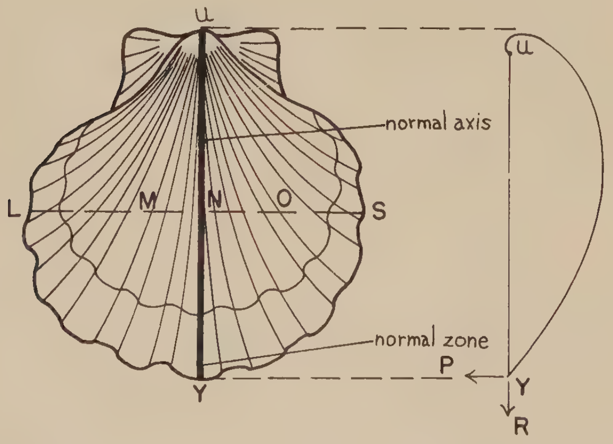

FIGURE 29.—Normal axis and the two growth components in the shell of scallop. LS—plane perpendicular to the plane of the generating curve; N—turning point of the concave side of the shell shown at right; M and O—auxiliary radii; P—transverse component; R—radial component; UY—normal axis. From Owen (1953).

GROWTH RINGS AND GROWTH RADII

Nearly 250 years ago Réaumur (1709) discovered that shells grow by the accretion of material secreted at their edges. Since that time this important observation has been confirmed by numerous subsequent investigations. The rings on the outer surfaces of a bivalve shell, frequently but incorrectly described as "concentric", represent the contours of the shell at different ages. Rings are common to all bivalves but are particularly pronounced on the flattened shells of scallops, clams, and fresh-water mussels. Depending on the shape of the shell, the rings are either circular or oval with a common point of origin at the extreme dorsal side near the umbo (figs. 30 and 31). The diagrams clearly show that the rate of growth along the edge of the shell is not uniform. It is greater along the radius, AD, which corresponds to the directive axis of Lison, and gradually decreases on both

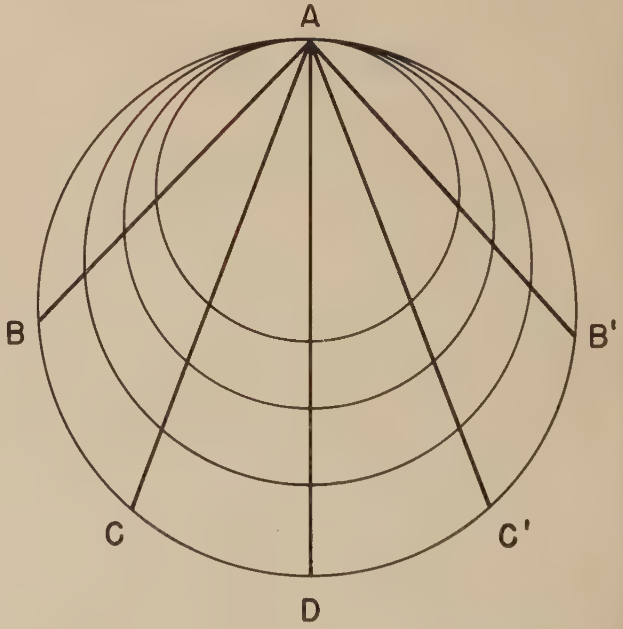

FIGURE 30.—Diagram of a circular bivalve shell of the kind represented in *Pecten*, *Anomia*, and young *C. virginica*. Radii extending from the umbo to the periphery of the generating curve are proportional to the rate of growth at the edge of a circular shell Radius AD corresponds to the directive axis of Lison.

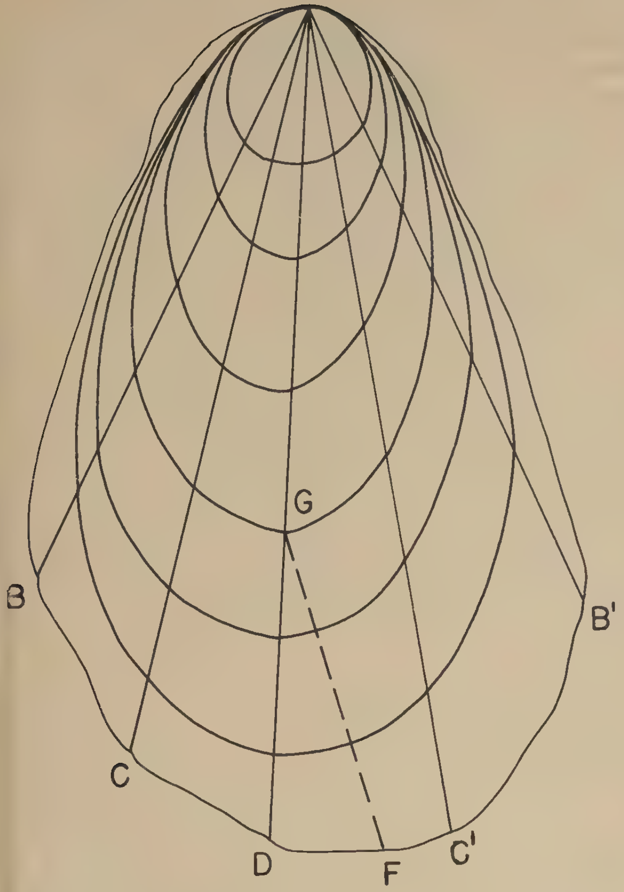

FIGURE 31.—Diagram of a shell of adult *C. virginica.* Radii extend from the umbo to the periphery of the generating curve. The principal axis AGF shows the change in the direction of growth at G. The length of radii is proportional to the rate of shell growth at the edge.

sides of it along growth radii AC, AB, and AC_1, AB_1.

Circular shells in *C. virginica* may be found only in very young oysters (fig. 32a). Within a few weeks after setting the shell becomes elliptical, and as elongation (increase in height) continues the principal vector of growth shifts to one side (fig. 32b).

A series of curves noticeable on round shells (fig. 32) clearly illustrate the differential rate of growth along the periphery of the valve, which increases in size without altering in configuration. Thompson (1942) found an interesting analogy between this type of growth, radiating from a single focal point (the umbo), and the theorem of Galileo. Imagine that we have a series of planes or gutters originating from a single point A (fig. 30) and sloping down in a vertical plane at various angles along the radii AB, AC, AC_1, and AB_1 which end at the periphery of a circle. Balls placed one in each gutter and simultaneously released will roll down along the vectors B, B_1, C, C_1, and D. If there is no friction or other form of resistance, all the balls will reach the periphery at the same time as the ball dropping vertically along AD. The acceleration along any of the vectors, for instance, AB, is found from the formula $t^2=2/g$ AD where t is time and g is acceleration of gravity.

A similar law, involving a more complex formula, applies to cases in which the generating curve is nearly elliptical, for instance, in the shells of adult oysters. The rate of growth at different sectors of the periphery of the shell obviously has nothing to do with the acceleration of gravity, but the similarity between the length of the radii which represent the rate of growth along a given direction of the shell and the acceleration along the vectors in the theorem of Galileo is striking. It appears reasonable to expect that the Galileo formula may be applicable to the physiological process taking place near the edge of the valve. One may assume, for instance, that the rate of physiological activities is affected by the concentration of growth promoting substances or by enzymes involved in the calcification of the shell and that these factors vary at different points of the mantle edge in conformity with Galileo's formula. Experimental exploration of the possibilities suggested by mathematical parallelism may be, therefore, profitable in finding the solution to the mystery of the formation of shell patterns.

CHANGES IN THE DIRECTION OF PRINCIPAL AXES OF SHELL

The principal axes of shells of *C. virginica* are not as permanent as they are in clams, scallops, and other bivalves in which the shape of the valves remains fairly constant and is less affected by environment than in the oyster. The plasticity of oysters of the species *Crassostrea* is so great that their shape cannot be determined geometrically (Lison, 1949). This inability to maintain a definite shape is probably the result of the sedentary living associated with complete loss of the power of locomotion.

In some species of oysters the shells are circular or nearly circular. In such cases the ratio of the height of the valve to its length is equal to

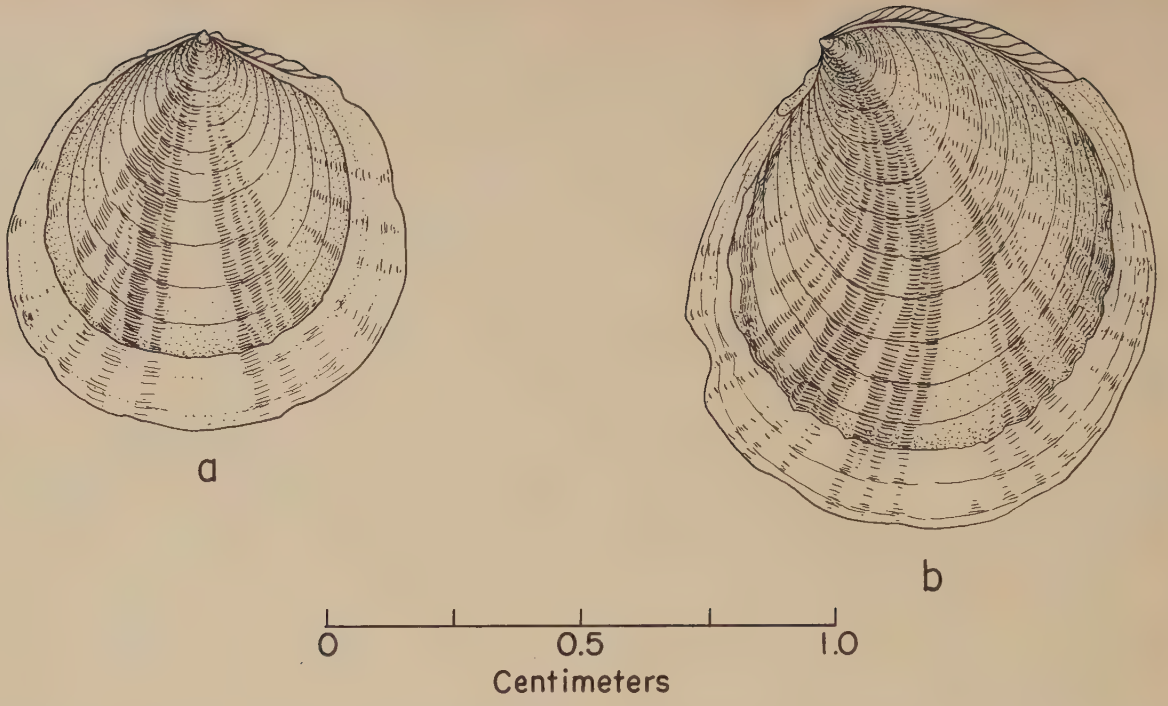

FIGURE 32.—Two small *C. virginica* growing attached to tar paper. Maximum dimension of shell: a—0.85 cm.; b—1.0 cm. At b the principal axis curves to the left.

1.0, as, for instance, in *C. rivularis* (fig. 8) and *O.* (*Alectryonia*) *megodon* Hanley (fig. 3) (Olsson, 1961). Oysters of the latter species from the Pacific Coast of Central and South America grow singly, in vertical position, cemented to the rocks by their left valves. The specimens I collected on Pearl Islands, Gulf of Panama, measured 17 to 18 cm. in height and 16 to 17 cm. in length. The European flat oyster, *O. edulis* (fig. 9) usually forms rounded shells in which the length exceeds the height. Small, noncommercial species, *O. sandwichensis* of the Hawaiian Islands and *O. mexicana* from the Gulf of Panama, are almost circular with the tendency to extend in length rather than in height. Crowded conditions under which these species thrive attached to rocks in a narrow tidal zone greatly obscure and distort the shape of their shells.

Small *C. virginica* growing singly on flat surfaces without touching each other are usually round (fig. 32). In a random sample consisting of 100 single small oysters (spat about 6 weeks old) varying from 5 to 15 mm. in height and growing on tar paper, the height/length ratio varied from 0.6 to 1.2. Nearly half of them (49 percent) were perfectly round (H/L ratio=1); in 30 percent the ratio was less than 1; and in 21 percent the length exceeded the height.

In small single oysters less than 10 mm. in height the principal (normal) axis of growth is clearly marked. All other radii symmetrically oriented on both sides of the principal axis are indicated by the pigmented bands on the surface of the shell. The newly deposited shell, discernible at the periphery of the oyster, forms a band which is wider at the ventral edge of the shell and slightly narrows anteriorly and posteriorly (fig. 32a). With the growth of the oyster its principal axis is shifted to the side, curves, and is no longer confined to one plane. The curvature of the valve becomes a turbinate-spiral. Gradually the oyster becomes slightly oval-shaped and asymmetrical.

The change in the direction of the principal axis of growth is not associated with the environment since it takes place only in some of the oysters growing under identical conditions. Occasionally oysters are formed in which the pigmentation along the principal axis is so pronounce that the dark band which marks its position ma be mistaken for an artifact (fig. 33) while the secondary axes are not visible. The shells o adult *C. virginica* usually curve slightly to th left (if the oyster is placed on its left valve an viewed from above). Frequently, however, in verted specimens are found in which the growt

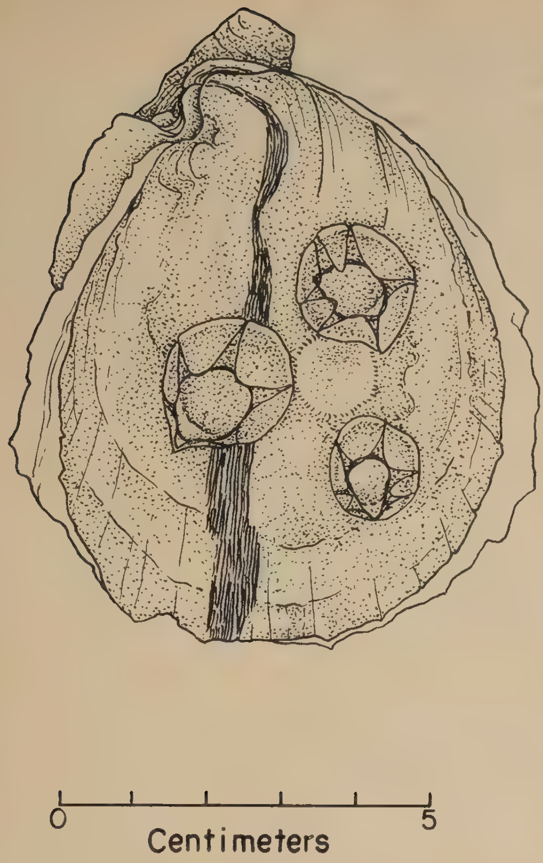

FIGURE 33.—Principal axis of growth of a *C. virginica* from Chatham, Mass., is deeply marked by a pigmented band.

has shifted into the opposite direction (fig. 34). The "normal" oyster (the right side of the figure) is curved to the left while in the inverted specimen, shown on the left of the figure, the shell curves to the right. Such "right-handed" oysters are probably common in all oyster populations since they were found in Texas, Chesapeake Bay, Narragansett Bay, and Great Bay, N.H. In every other respect the inverted specimens are normal and had typically cupped left valves with well-developed grooved beaks. There is no evidence that inversion was caused by mechanical obstruction or some unusual position on the bottom.

Complete inversion in bivalves was described by Lamy (1917) for *Lucina*, *Chama*, and several species of the subgenus *Goodallia* (fam. Astartidae). It consisted in the appearance of structures, typical for the right valve, on the left valve and vice versa. In the case of *C. virginica* the structural elements remain unaffected and the inversion is limited to the contours of the valves.

The once established principal axis of growth does not always remain unchanged. Occasionally old oysters are found in which the direction of growth had undergone sudden changes of about 90°. The change shown in figure 35 took place when both oysters were about 6 to 7 years old.

The instability of the principal axis of growth may be even more pronounced. My collection has an oyster (*C. virginica*) found on the banks of a lagoon near Galveston, Tex., in which the principal axis, clearly indicated by pigmented bands on the surface of the valves, changed its direction at the end of each growing period. The resulting zigzag line is clearly visible in the specimen (fig. 36).

DIMENSIONAL RELATIONSHIPS OF SHELL

Shape of a bivalve shell is often expressed as a ratio between its height and length or by some other numerical index. Lison (1942) pointed out that the shape of an oyster shell cannot be expressed in precise geometrical terms, presumably because of its great variability. The "index of shape" determined as a ratio of the sum of height and width of a shell to its length was used by Crozier (1914) in studying the shells of a clam, *Dosinia discus*. For the mollusks ranging from 2 to 7 cm. in length collected near Beaufort, N. C. this index varied from 1.24 to 1.28 indicating that the increase of the species in height and width was directly proportional to the increase in length. Such regularity is not found in the shells of adult *C. virginica* taken at random from commercially exploited bottoms. For the entire range of distribution of this species in the Atlantic and Gulf states the index of shape varied from 0.5 to 1.3. The histogram (columns in figure 37) shows nearly normal frequency distribution with the peak of frequencies at 0.9. No significant differences were found in the index of shape in the northern and southern populations of oysters examined separately. The boundary between the two groups was arbitrarily drawn at the Virginia-North Carolina line. The two curves connecting the frequency points on figure 37 indicate that in the southern population the index of shape extends from 0.5 to 1.3, while in the northern oysters it varies from 0.6 to 1.2. The difference is probably not very significant, but it may be due to a greater percentage of wild oysters on commercially exploited natural bottoms of the southern states.

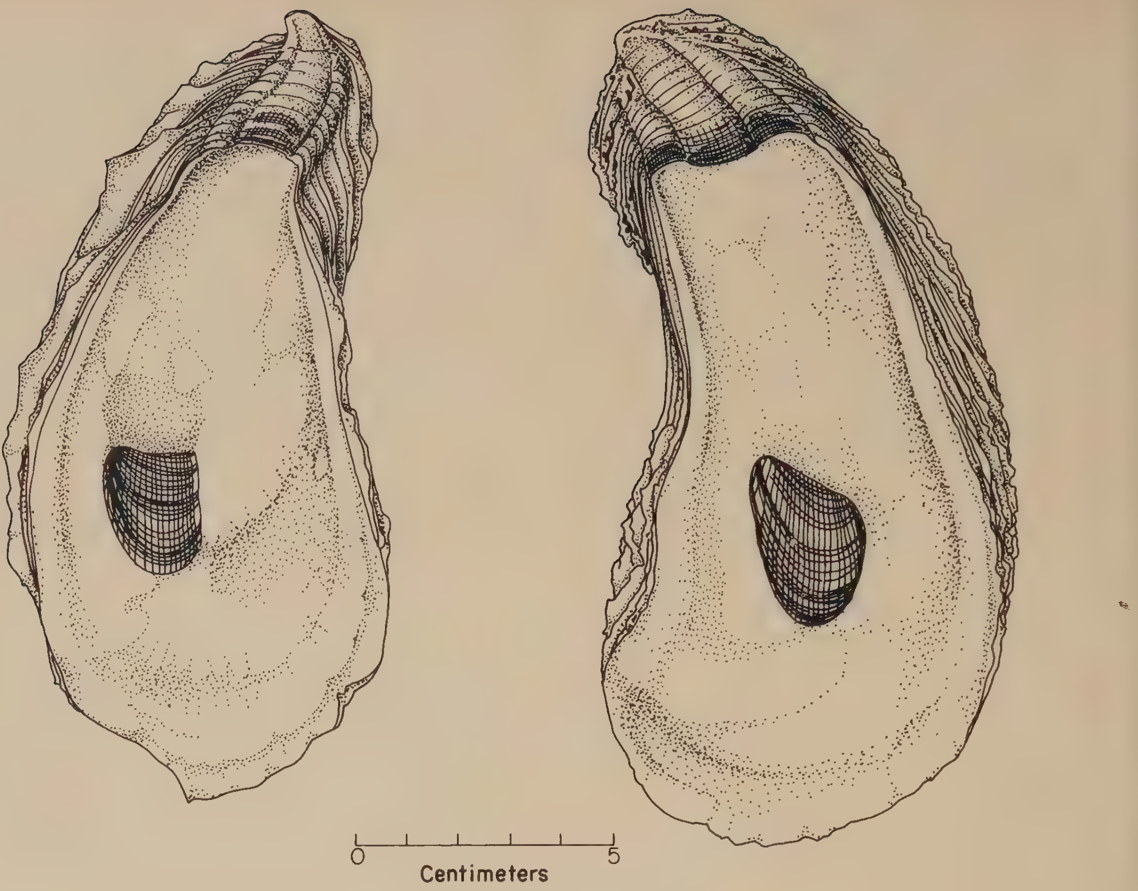

Figure 34.—Left valves of the two large *C. virginica* from Narragansett Bay, R.I. On the right is a "normal" oyster; its shell curves to the left. On the left side is an inverted oyster; its shell curves to the right.

Most of the oysters from the North Atlantic and Chesapeake states were taken from bottoms on which oysters are regularly planted for cultivation. There are no significant differences in the mean, mode, and median of the two groups (table 1). Contrary to the conditions found by Crozier in *Dosinia discus*, the "index of shape" of *C. virginica* is highly variable.

SHELL AREA

Information regarding the approximate area of an oyster shell of known height may be useful to oyster growers who want to determine in advance what percentage of the bottom area set aside for planting will be covered by oysters of known size. Since the oystermen usually know the number of oysters of various sizes needed to make up a bushel, the information given below may be used in determining in advance whether the area of the bottom is sufficient to provide space for their additional growth.

Table 1.—*Index of shape* $\left(\frac{\text{height}+\text{width}}{\text{length}}\right)$ *of oysters taken by commercial fishery*

Locality	Mean	Standard deviation	Mode	Median
Northern grounds	0. 87	0. 05	0. 94	0. 09
Southern grounds	0. 87	0. 02	0. 94	0. 9

It is self-evident that the area of the valve increases proportionally to the increase in its linear dimensions. For determining the area a piece of thin paper was pressed against the inner surface of the right (flat) valve and the outlines were drawn with pencil. The area was measured with a planimeter. The outlines of small shells were placed over graph paper and the number of millimeter squares counted.

The relationship between the height and shell area (fig. 38) is represented by an exponential curve of a general type $y=ax^b$ which fits many empirical data. The y in the formula is the shell area, and the x is the height. The parabolic nature of the curve is demonstrated by the fact

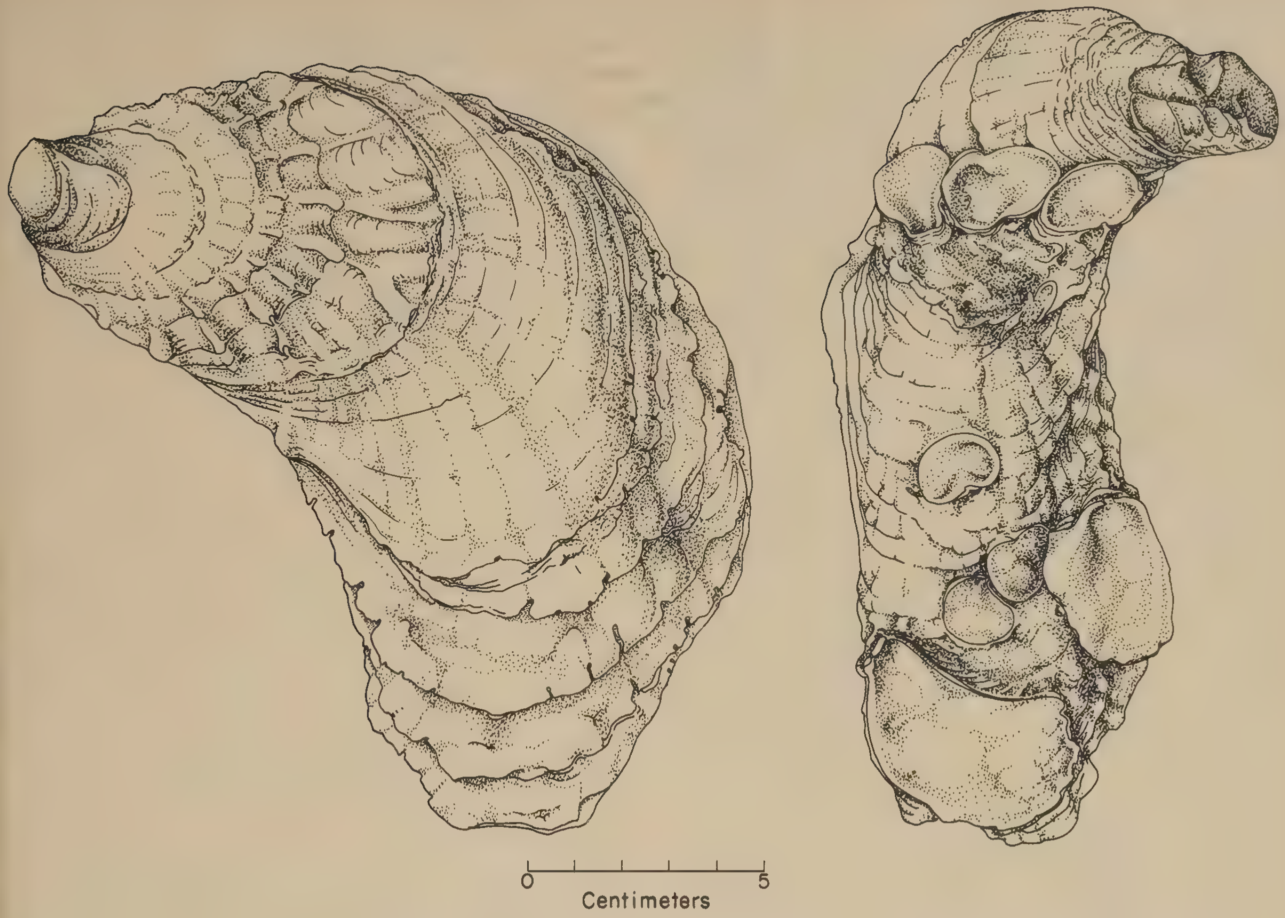

Figure 35.—Two upper (right) shells of old *C. virginica* from Chesapeake Bay (left) and Matagorda Bay, Tex. (right). The direction of growth changed suddenly about 50° to the left in the Chesapeake oyster and about 75° to the right in the Texas oyster.

that the log/log plot (fig. 39) fits a straight line. The numerical values of factors a and b were found to be equal to 1.25 and 1.56 respectively. The formula reads, therefore, $y=1.25x^{1.56}$. As a convenience to the reader who may be interested in finding directly from the curve the average area occupied by a shell of a given height, the data computed from the equation can be read from the curve in figure 38. The measurements are given both in centimeters and inches. The data refer to the random collection of live oysters from the coastal areas between Prince Edward Island, Canada, and the eastern end of Long Island Sound (table 2).

The relationship between the height and area of the upper valve of *C. virginica* is in agreement with the findings of other investigators (Newcombe, 1950; Nomura, 1926a, 1926b, 1928) who concluded that in several marine and fresh-water bivalves and gastropods the dimensional relationships can be adequately expressed by the formula of heterogenic growth, $y=bx^k$. According to Nomura's (1926a) interpretation of the growth of the clam *Meretrix meretrix*, the constant b in this formula represents the effect of the environment while k is a factor of differential growth. Nomura's conclusions may be applicable to other bivalves, and if confirmed by further studies this method may become useful for quantitative de-

Table 2.—*Height and shell area of northern oysters computed by using the equation* $y=1.25x^{1.56}$

Height		Area	
Cm.	*Inches*	*Cm.*^{2}	*In.*^{2}
5	1.97	15.4	2.39
6	2.36	20.5	3.18
8	3.15	32.3	5.01
10	3.94	45.4	7.04
12	4.72	60.3	9.35
14	5.51	76.7	11.9
16	6.30	94.5	14.6
18	7.09	113.5	17.6
20	7.87	133.8	20.7

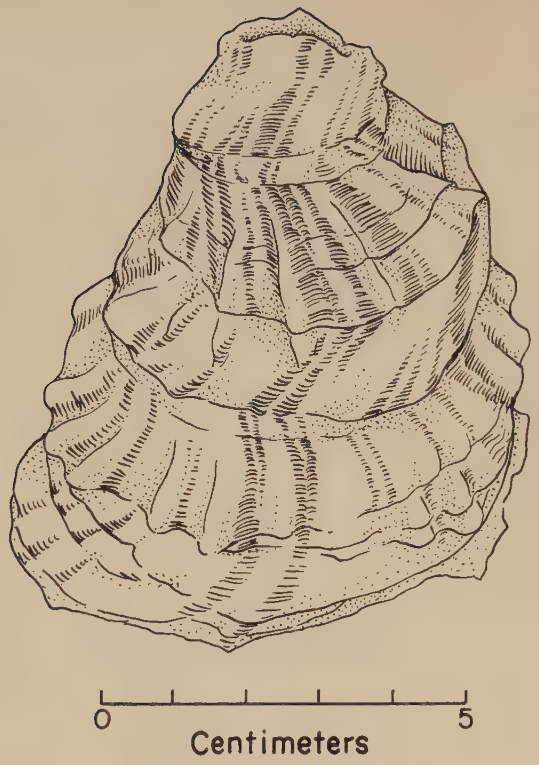

FIGURE 36.—Shell of an adult *C. virginica* showing periodic changes in the direction of the principal axis of growth. Note the zigzag line of pigmented bands in the middle of the valve. Actual dimensions: 8.5 by 6 cm. (3.25 by 2.5 inches).

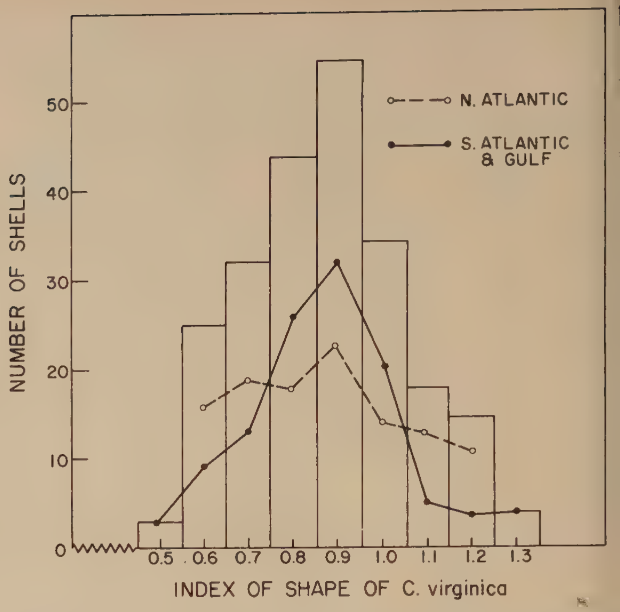

FIGURE 37.—Histogram of the distribution of the index of shape $\frac{\text{(height+width)}}{\text{length}}$ of shells of *C. virginica* from the Atlantic Coast. Frequency distribution of the index of North Atlantic oysters (open circles) and South Atlantic oysters (points) are shown by two separate curves.

terminations of the effect of local conditions on growth and shape of shells.

CHALKY DEPOSITS

The glossy, porcelainlike inner surface of an oyster shell is frequently marred by irregularly shaped white spots which consist of soft and porous material of different appearance and texture than the surrounding shell substance. These areas are called "chalky deposits". They are very common in *C. virginica* and *O. edulis.* Since the first record of their presence in edible oysters made by Gray (1833) they have been mentioned frequently by many biologists. Recent review of the literature on the subject is given by Korringa (1951).

The exact location of chalky deposits is of interest since some speculations regarding their role and origin are based on the position they occupy on the shell. Orton and Amirthalingam (1927) assumed that chalky material is formed in the places where the mantle loses contact with the shell. No experimental evidence in support of this explanation was presented by the authors or by Ranson (1939–41), who fully accepted the theory without making additional studies and stated positively that chalky deposits are formed wherever there is a local detachment of the mantle from the valve.

Considering the possibility that the mantle may be more easily detached from the valve if the oyster is placed with its lower (cuplike) side uppermost, Korringa (1951) made a simple field experiment. In one tray he placed 25 medium sized *O. edulis* in their normal position, with their cupped valves undermost; the other tray contained an equal number of oysters resting on their flat valves. At the end of the growth season he observed no significant differences in the deposition of shell material in the oysters of the two groups.

To determine whether chalky deposits are formed in places of partial detachment of the mantle, I performed the following experiment: Small pieces of thin plastic about 1 cm.^{2} were bent as shallow cups and introduced between the mantle and the shell of *C. virginica.* In 10 oysters the cups were inserted with the concave side facing the mantle, in another 10 oysters the position of the cup was reversed, i.e., the concave side faced the valve. The oysters were kept for

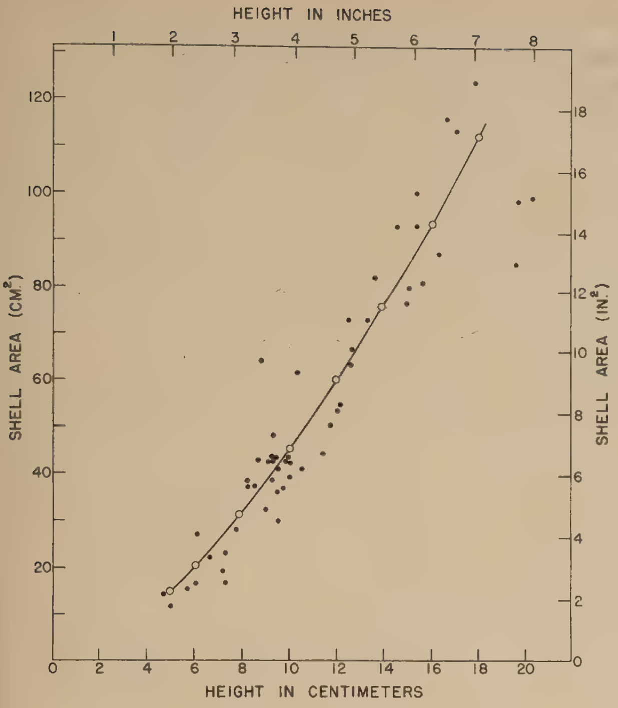

FIGURE 38.—Shell area in cm.² plotted against height of shells in cm. Inch scales are on top and on the right.

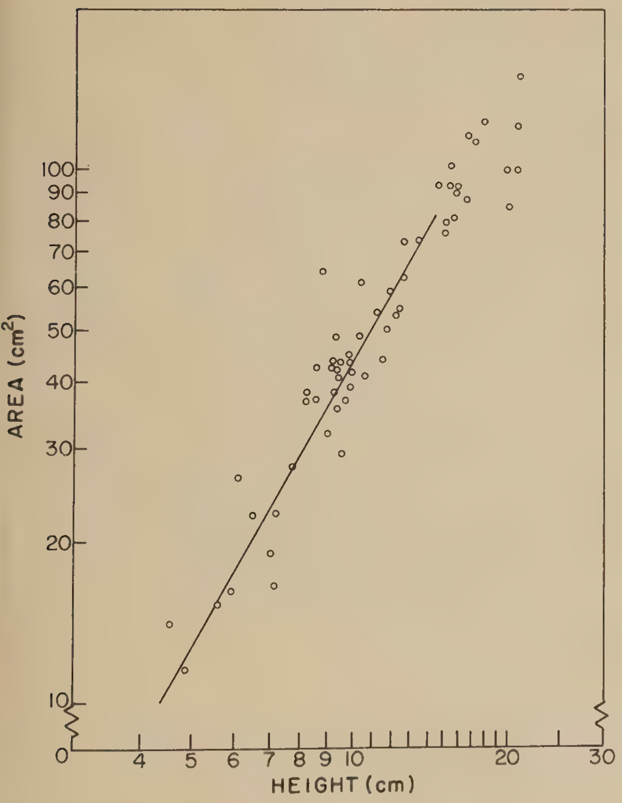

FIGURE 39.—Logarithmic plot of shell area against shell height.

55 days in running sea water in the laboratory. During this time they fed actively and had considerable shell growth along the margin of the valves. After their removal from the shells the cups were found to be covered with hard calcite deposits on the sides facing the mantles. No chalky material was found on cups or on the surface of valves adjacent to the area of insertion. On the other hand, conspicuous chalky areas were formed along the edge of the shell in places where the opposing valves were in close contact with each other (fig. 40). It is clear from these observations that the detachment of the mantle from the inner surface of the shell does not result in the deposition of chalky material and that such deposits may be laid in the narrowest space of shell cavity where the two valves touch each other.

Suggestions that chalky deposits result from secondary solution of calcium salts of the shell (Pelseneer, 1920) or that their formation is somehow related to the abundance of calcareous material in the substratum (Ranson, 1939–41,

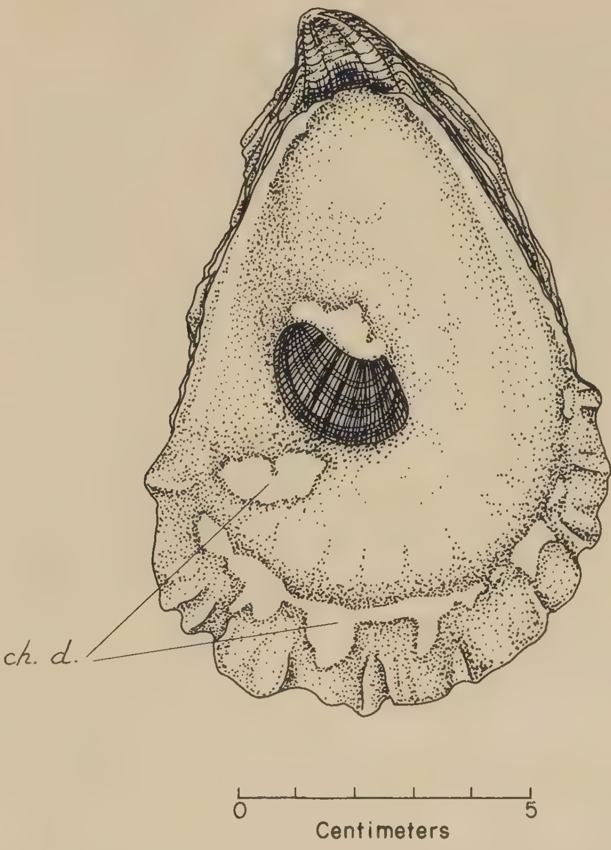

FIGURE 40.—Chalky deposits (ch. d.) on the newly formed shell at the edge of the valve, and near the muscle attachment.

1943) are not supported by evidence. The inner surface of bivalve shells may become slightly eroded due to the increased acidity of shell liquor when the mollusk remains closed for a long time, but the erosion is, however, not localized; it occurs over the entire shell surface. As to the effect of the abundance of lime in the substratum on the formation of chalky deposits, one must remember that the concentration of calcium salts dissolved in sea water is fairly uniform and that calcium used for building of shells is taken directly from the solution (see p. 103). Under these conditions the abundance of calcium carbonates in bottom deposits cannot have any effect on the formation of shell.

Chalky areas of shell do not remain unchanged. They become covered by hard substance and in this way they are incorporated in the thickness of the valves (fig. 41).

Korringa's theory (1951) that the oyster deposits chalky material ". . . when growing older, in its efforts to maintain its efficiency in functioning" and that ". . . where possible the oyster always uses soft porous deposits when quite a lot of shell volume has to be produced . . ." is based on the assumptions: (1) that chalky deposits most frequently develop in the area posterior to the muscle attachment, (2) that the layers of chalky material are more numerous in cupped than in flat oysters, (3) that in the area of the exhalant chamber (in the posteroventral quadrant of the shell) the oyster attempts to decrease the distance between the two valves by rapid deposition of shell material, and (4) that chalky material is used by the oyster "as a measure of economy, as a cheap padding in smoothing out the shell's interior." The validity of these assumptions with reference to *C. virginica* was tested by studing the relative frequency of the occurrence of chalky deposits on the left and right valves and by estimating the extent of these deposits in different parts of the valves. The collection of shells studied for this purpose comprised several hundred adult specimens from various oyster beds along the Atlantic and Gulf coasts. For determining the distribution of chalky areas the inner surface of the valves was arbitrarily divided into four quadrants shown in figure 42 and designated as follows: A—dorsoposterior; B—dorsoanterior; C—ventroposterior; and D—ventroanterior. The following five classes corresponding to the degree of the development of chalky deposits in each quadrant were established:

No deposits within the quadrant	0
1 to 25 percent of the area covered with deposits	1
26 to 50 percent of the area covered with deposits	2
51 to 75 percent of the area covered with deposits	3
76 to 100 percent of the area covered with deposits	4

With a little practice it was easy to select the correct class by visual examination. The first question was whether there is any difference in the frequency of occurrence and extent of chalky deposits on right and left valves. For this purpose the entire surface of the valve was examined and classified. Chalky deposits were found as often on the right as on the left valve of *C. virginica*. This is shown in table 3 which summarizes the observations made on 472 shells collected at random at oyster bottoms along the

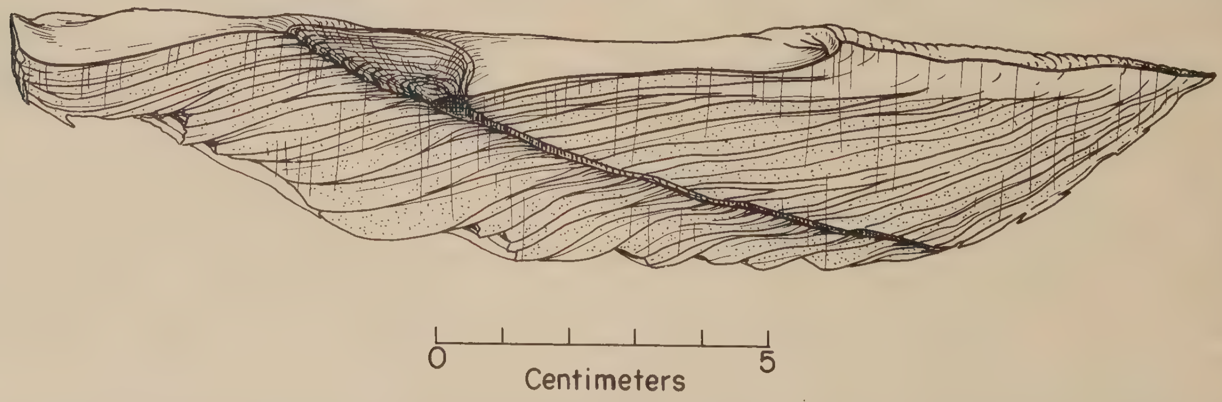

FIGURE 41.—Left valve of an old *C. virginica* cut along the principal axis of growth. Chalky areas on both sides of the hypostracum (dark platform for the attachment of the adductor muscle) are enclosed in the thin layers of hard crystalline material. Hinge on the right. Natural size.

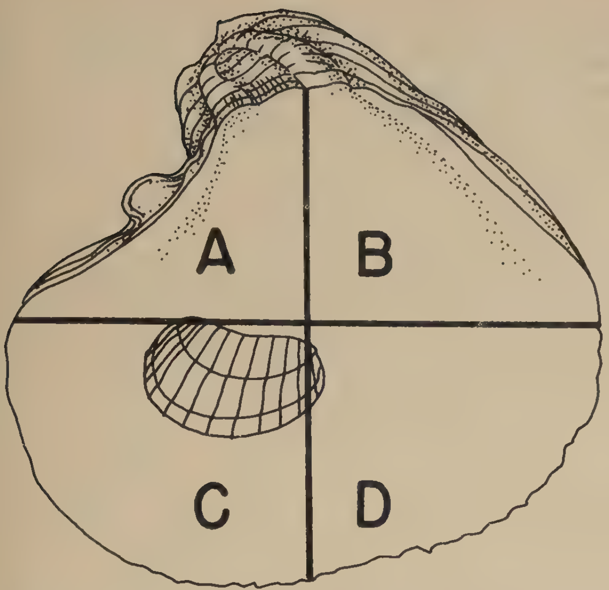

FIGURE 42.—Four arbitrary quadrants of the inner surface of shell used for estimating the distribution and extent of chalky deposits.

Atlantic Coast from Long Island Sound to Georgia. Nearly one-half of the total number of valves examined (48 percent of left and 53 percent of right valves) were free of the deposits. (The percentage of oysters without chalky deposits was not determined because in many shells of the collection the valves had separated and could not be arranged in pairs.) In about 25 percent of the total number of shells the chalky deposits cover less than one-quarter of the valve area. Larger deposits occurred in diminishing number of shells; those covering more than three-quarters of available space (class 4) comprised less than 3 percent of the total number examined.

There was no particular area on the valve surface where chalky deposits were formed more often than in any other place. The differences in the frequency of their occurrence in different quadrants of a valve were not significant.

In *O. edulis*, according to Korringa, chalky deposits form more often in deep (cupped) shells than in flat ones and can be found principally in the area in front of the cloaca, quadrant C according to our terminology. No such differences in the place of formation or in the type of shell could be observed in *C. virginica.*

TABLE 3.—*Percent of valves of* C. virginica *with chalky deposits*

Item	Area of valve covered by chalky deposits			
	Class 1 (1–25 percent)	Class 2 (26–50 percent)	Class 3 (51–75 percent)	Class 4 (76–100 percent)
Left valve	25.9	13.6	9.8	2.8
Right valve	24.9	12.1	8.4	1.5

From the observations on oysters of Prince Edward Island, Medcof (1944) concluded that chalky deposits are normal parts of shells and that they have "functional importance" in preserving "a size relationship between meats and shell cavity" and in regulating "the curvature of the inner face of the shell throughout the oyster's life." There could be no argument about the first conclusion that chalky deposits are normal parts of the oyster shell. The fact that they appear during the first weeks of the oyster's life confirms this statement. The second conclusion that they preserve the curvature of the shell is impossible to prove without careful study of a large number of shells. In comparing the contours of the shells of New England and Chesapeake Bay oysters with and without chalky deposits, I failed to notice any significant difference between the two groups.

Japanese investigators (Tanaka, 1937, 1943) found great variability in the distribution of chalky deposits in *C. gigas* and *C. futamiensis.* Large porous areas may be found in the shells of these species near the anus, in front of the labial palps, or near the gonads. There seems to be no evidence that they occur primarily in one particular place of the valve. These observations agree with my observations on *C. virginica.*

CHAMBERING AND BLISTERS

The French word "chambrage" or chambering has been used by European biologists to describe shallow cavities, mostly in the cupped valves of *O. edulis.* The cavities are usually filled with sea water and putrified organic material. In the museum specimens these spaces are dry and filled with air. Sometimes only one chamber is found, but occasionally an entire series of cavities may be present. The chambers may be invaded by tube-forming annelids living in the oyster (Houlbert and Galaine, 1916a, 1916b). The successive layers of shell material in the chamber are not in contact with each other but surround an empty space. This gives the impression that the body of the oyster had shrunk or retracted and occupies only a small portion of shell space. This view is generally accepted by European oyster biologists

(Korringa, 1951; Orton, 1937; Orton and Amirthalingam, 1927; Worsnop and Orton, 1923), who agree that chambering is caused by the shrinkage of the body, withdrawal of shell-forming organ, and deposition of partitions. Salinity changes were suggested by Orton as one of the principal causes of chambering, and shrinkage due to spawning was also considered by Korringa as a probable factor. These conditions have not been reported for *C. virginica*. I did not find any evidence that chambers or blisters in the American oyster are associated with shrinkage or other body changes.

It is interesting to add that some taxonomists of the middle of the past century (Gray, 1833; Laurent, 1839a, 1839b) were so puzzled by the presence of chambers that they compared chambered oyster with *Nautilus* and even suggested the possibility of some family relation between the latter genus and *Ostrea!*

An interesting shell structure consisting of a series of chambers near the hinge end is found in the Panamanian oyster, *O. iridescens*. The location of chambers and the regularity at which they are formed as the shell grows in height can be seen in figure 43 representing a longitudinal section of the valve made at a right angle to the hinge. This type of chambering is obviously a part of a structural plan of the shell and is not a result of an accidental withdrawal of the oyster body or of an invasion by commensals. Arch-forming septae of the chambers apparently contribute to the strength of the hinge and at the same time require relatively small amounts of building material. What advantage *O. iridescens* obtains from this type of structure is of course a matter of speculation.

Chambers found in *C. virginica* consist of irregular cavities containing mud or sea water. Such formations are called blisters. Blisters can be artificially induced by inserting a foreign object between the mantle and the shell (see p. 105). They are also caused by the invasion of shell cavity by *Polydora* (see p. 422) or by perforations of the shell by boring sponges and clams (p. 420).

STRUCTURE OF SHELL

For more than a hundred years the structure of the molluscan shell was an object of research by zoologists, mineralogists, and geologists. Several reviews of the voluminous literature (Biedermann, 1902a, 1902b; Bøggild, 1930; Cayeux, 1916; Haas, 1935; Korringa, 1951; Schenck, 1934; Schlossberger, 1856) deal with the problem from different points of view. Recently these studies have been extended by the use of X-ray and electron microscope. The methods, especially those of electron microscopy, opened entirely new approaches particularly with reference to the structure of the organic constituents of the shell (Grégoire, 1957; Grégoire, Duchâteau, and Florkin, 1950, 1955; Watabe, 1954).

Terminology of molluscan shells is somewhat confusing depending whether the emphasis is placed on morphological, crystallographical, or mineralogical properties. The names of different

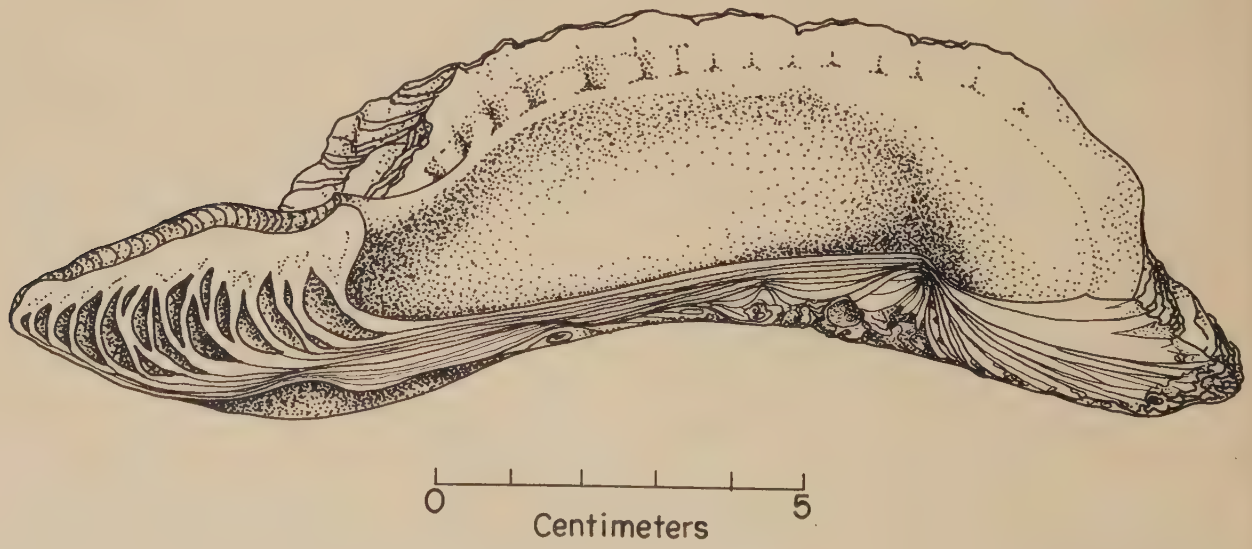

FIGURE 43.—Shell of *O. iridescens* cut at right angle to the hinge. Note a series of empty chambers at the hinge area. Specimen from the Gulf of Panama.

layers of shell described in this chapter are those which are found in more recent biological publications (Korringa, 1951; Leenhardt, 1926).

The shell of the oyster consists of four distinct layers: periostracum, prismatic layer, calcite-ostracum, and hypostracum. The periostracum is a film of organic material (scleroprotein called conchiolin), secreted by the cells located near the very edge of the mantle. The periostracum is very poorly developed in *C. virginica* and cannot be found in old shells. It covers the prismatic layer which can be best studied by removing from the edge of an oyster a small piece of newly formed shell. Microscopic examination reveals that the prismatic layer is made of single units shown in figure 44. Each prism consists of an aggregate of calcite crystals (Schmidt, 1931) laid in a matrix of conchiolin which after the dissolution of mineral constituents in weak hydrochloric acid retains the general configuration of the prisms (fig. 45). The double refraction of the walls of empty prisms is pronounced and causes slight iridescence noticeable under the microscope. In a well-formed layer the prisms are wedge-shaped and slightly curved (fig. 46). Conchiolin adhering to the prisms can be destroyed by boiling in potassium hydroxide solution and the prisms separated (Schmidt, 1931). Their shape and size are very variable.

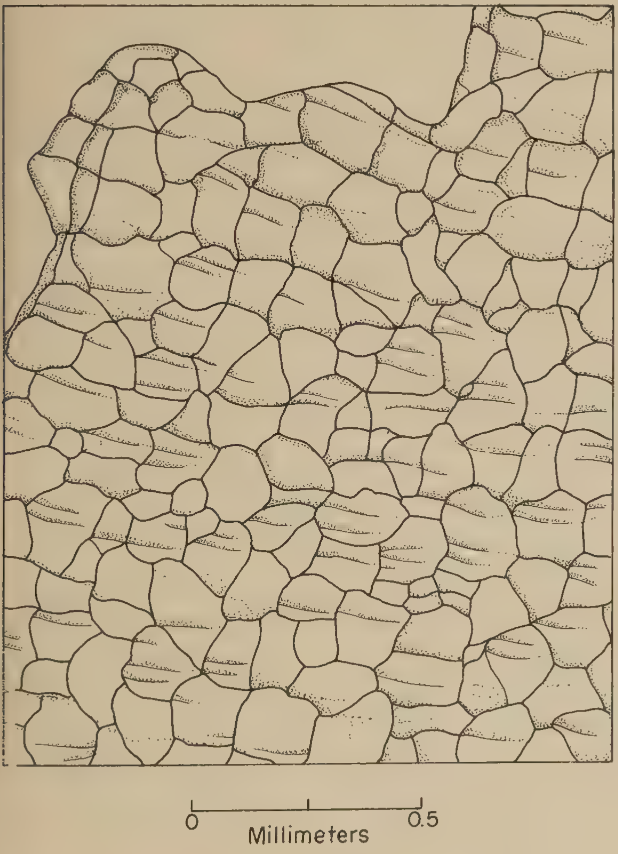

FIGURE 44.—Prismatic layer at earlier stages of calcification. *C. virginica.*

The optical axes of the prism are, in general, perpendicular to the plane of the prismatic layer, but in places they are irregularly inclined toward it.

Calcite-ostracum, called also a subnacreous layer (Carpenter, 1844, 1847), makes up the major part of the shell. The layer consists primarily of foliated sheets of calcite laid between thin membranes of conchiolin. The separate layers are irregularly shaped with their optical axes in accidental position (Bøggild, 1930). In a polished, transverse section of the shell of *C. virginica* the folia are laid at various angles to the surface (fig. 47). This layer is frequently interrupted by soft and porous chalky deposits (upper two layers of fig. 47) which appear to consist of amorphous material. It can be shown, however, that chalky deposit is formed by minute crystals of calcite oriented at an angle to the foliated lamellae of the hard material.

Hypostracum is a layer of shell material under the place of the attachment of the adductor muscle. In the shells of *C. virginica* the layer is pigmented and consists of aragonite (orthorhombic calcium carbonate, $CaCO_3$).

For many years oyster shells were considered to be composed entirely of calcite (Bøggild). Recently Stenzel (1963) has discovered that on each valve of an adult *C. virginica* aragonite is present as padding of the muscle scar, in the imprint of Quenstedt's muscle, and in the ligament.

As the oyster grows the adductor muscle increases in size and shifts in the ventral direction. The new areas of attachment become covered with aragonite while the older, abandoned parts are overlaid with the calcite. The progress of the muscle from hinge toward the ventral side can be clearly seen on a longitudinal section of the shell where it can be easily distinguished by its darker color and greater hardness of the secreted material (fig. 48).

ORGANIC MATERIAL OF THE SHELL

After the removal of mineral salts of the shell by weak acids or by chelating agents, such as sodium versenate, the insoluble residue appears in the

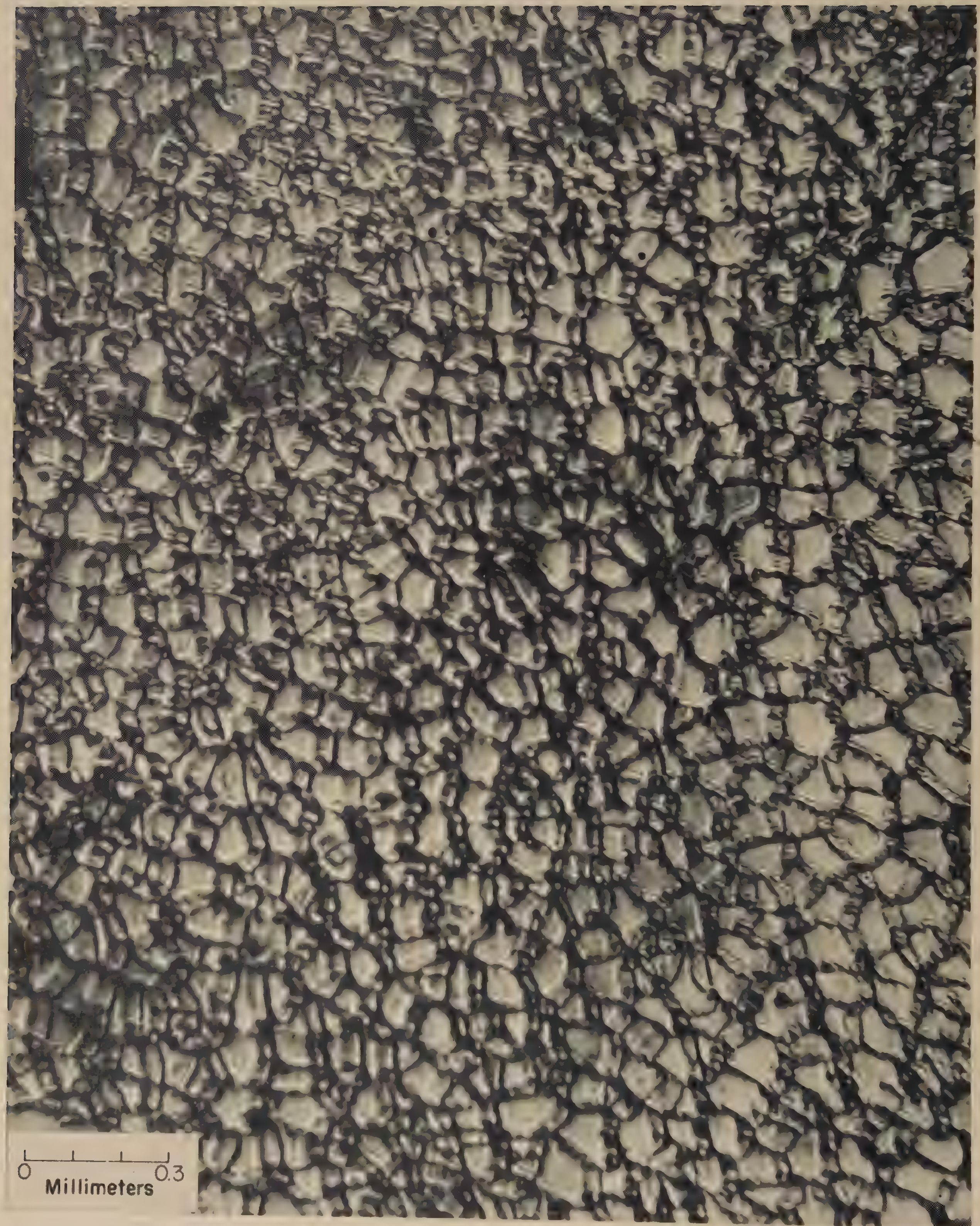

FIGURE 45.—Photomicrograph of a thin piece of prismatic layer after the dissolution of calcium carbonate in weak acid, *C. virginica.* The walls retain the shape of the prisms and are iridescent.

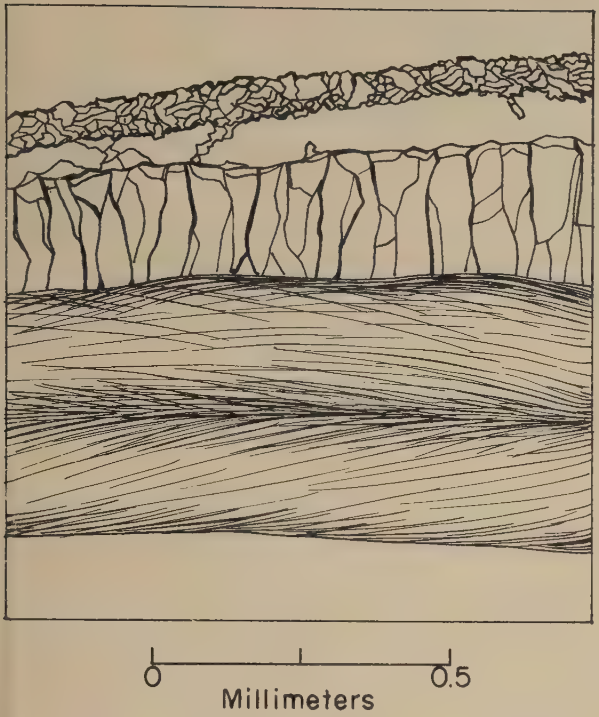

FIGURE 46.—Cross section of a piece of young shell of *C. virginica* (mounted in bakelite and ground on a glass wheel with carborundum, about 80 x). Periostracum (top line), prismatic layer (middle), and calcite-ostracum (lower).

form of thin, homogenous sheets of organic material kept together like pages of a book. This substance, discovered in 1855 by Fremy, is known as conchiolin. The name is applied to the organic material insoluble in water, alcohol, ether, cold alkaline hydroxides, and dilute acids. In the literature it appears also under the names of conchin, periostracum, epidermis, and epicuticula. Conchiolin is a scleroprotein, the structural formula of which has not yet been determined. The elementary analysis of conchiolin of *O. edulis* (Schlossberger, 1856) is as follows: H, 6.5 percent; C, 50.7 percent; N, 16.7 percent. Wetzel (1900) found that conchiolin contains 0.75 percent of sulfur and Halliburton (quoted from Haas, 1935) assigned to it the following formula: C_{30}, H_{48}, N_9, O_{11}, which also appears in the third edition of "Hackh's chemical dictionary" (Hackh, 1944). Similarity of conchiolin to chitin leads many investigators to an error in ascribing chitinous composition to structures which were found insoluble in alkaline hydroxides and dilute acids. Thus, the presence of chitin was reported in the shell and ligament of *Anodonta*, *Mya*, and *Pecten* (Wester, 1910). The application of the Schulze's test for chitin (intense violet coloration after treatment for 24 hours in diaphanol [chlorodioxyacetic acid], followed by a solution of zinc chloride and iodine), does not confirm these findings (Lison, 1953).[4]

To the naked eye and under the light microscope the conchiolin appears as amorphous, viscous and transparent material which hardens shortly after being deposited. Using the electron microscope technique, Grégiore, Duchâteau, and Florkin (1955) found that the conchiolin of gastropods and bivalves consists of a fine network with many meshes of irregular shape and variable dimensions. This is, however, not the case in oyster shells. Conchiolin of the genus *Ostrea* lacks meshes and under the electron microscope is of uniform appearance (personal communication by Grégoire).

Cross sections of decalcified shells of *C. virginica* show a distinct difference between the staining properties of the conchiolin of the prismatic and calcite-ostracum layers. On the cross sections of shell shown in figure 49 the two parts can be recognized by the typical foliated appearance of the calcite-ostracum and the meshlike structure of the prismatic layer. In the preparation stained with Mallory triple dye the organic matter of the walls of the prisms are stained reddish-brown while the foliae of the calcite-ostracum are bluish. Differential staining indicates the difference in the chemical composition of the two parts.

The amount of conchiolin in the oyster shell was studied by several investigators. As early as 1817 Brandes and Bucholz estimated that organic material of the shell constitutes about 0.5 percent of the total weight. Schlossberger (1856) found 6.3 percent of organic matter in the prismatic layer of the oyster but only from 0.8 to 2.2 percent in the calcite-ostracum. According to Douvillé (1936), the albuminoid content of the oyster shell is 4.8 percent.

According to the determinations made by A. Grijns for Korringa (1951), the conchiolin content of the prismatic layer of *O. edulis* varied from 3.4 to 4.5 percent against the 0.5 to 0.6 percent in the calcite-ostracum. The conchiolin content was calculated from the percentage of N (by Kjeldahl method) multiplied by 6.9. The results of my determinations of the weight of organic material

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[4] Inasmuch as the same reaction is obtained with cellulose and tunicine, additional tests should be made using Lugol solution and 1 to 2 per cent sulphuric acid (H_2SO_4). With this test chitin is colored brown, while cellulose and tunicine are blue.

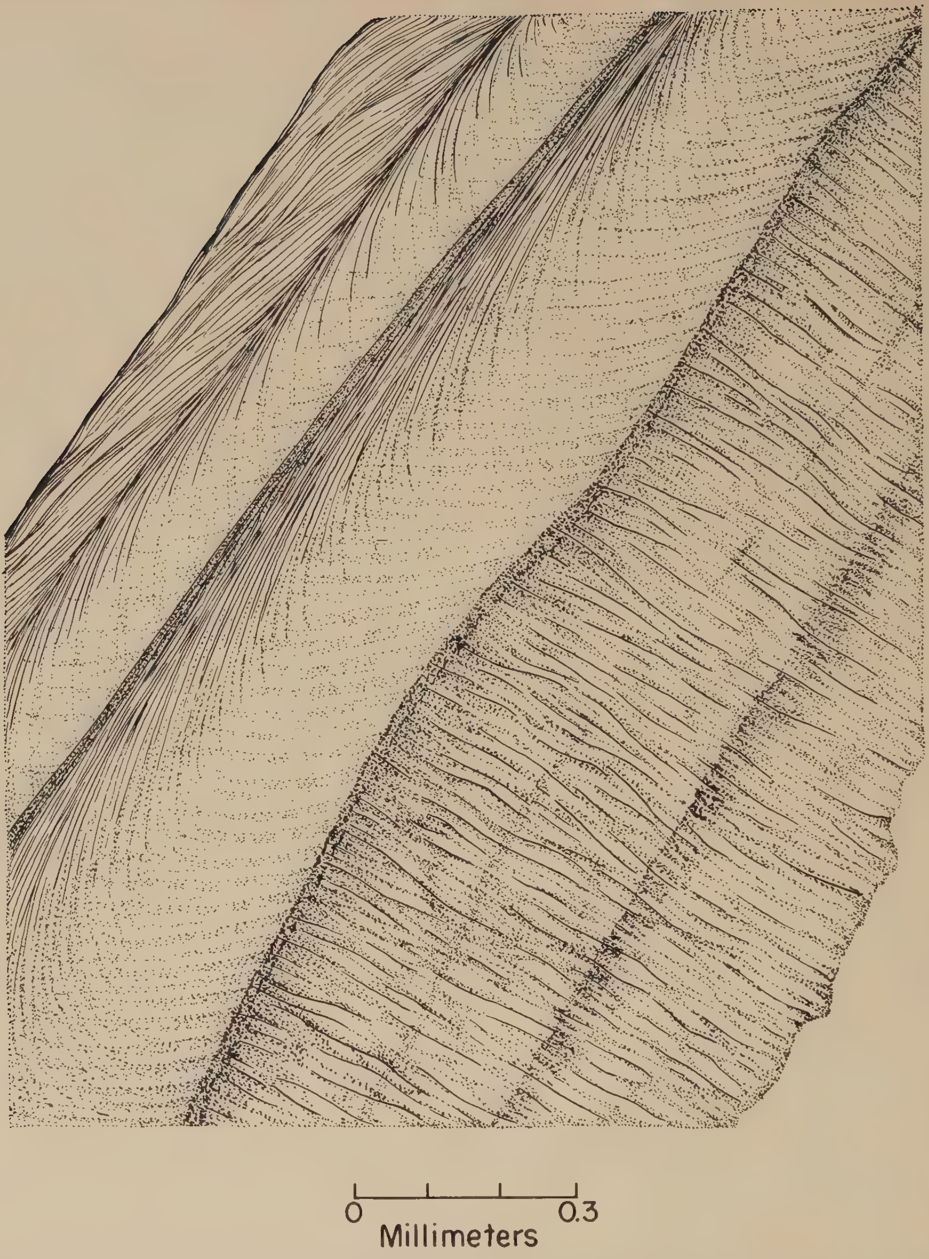

FIGURE 47.—Cross section of the shell of adult *C. virginica* embedded in bakelite and polished on a glass wheel with carborundum. Two upper layers consist of chalky deposits.

after decalcification of the calcite-ostracum of *C. virginica* shells from Long Island Sound and Cape Cod waters are in agreement with those given for *O. edulis*. The content of conchiolin in my samples varied from 0.3 to 1.1 percent with the mode at 0.6 percent. For these analyses 23 pieces of shell were taken from 16 adult oysters not damaged by boring sponge. The samples varied in weight from 0.5 to 15 g.

Higher percentage of conchiolin in the prismatic layer may be expected because this layer represents the new growth of shell which has not yet completely calcified.

The role played by conchiolin in the deposition of calcium salts in the form of calcite or aragonite presents a very interesting problem which has not yet been solved. Recent electron microscope studies of pearl oyster shells made by Grégoire show that the organic material in which aragonite crystals are laid (Grégoire, Duchâteau, and Florkin, 1950) is arranged as a series of bricklike structures. No such arrangement has been de-

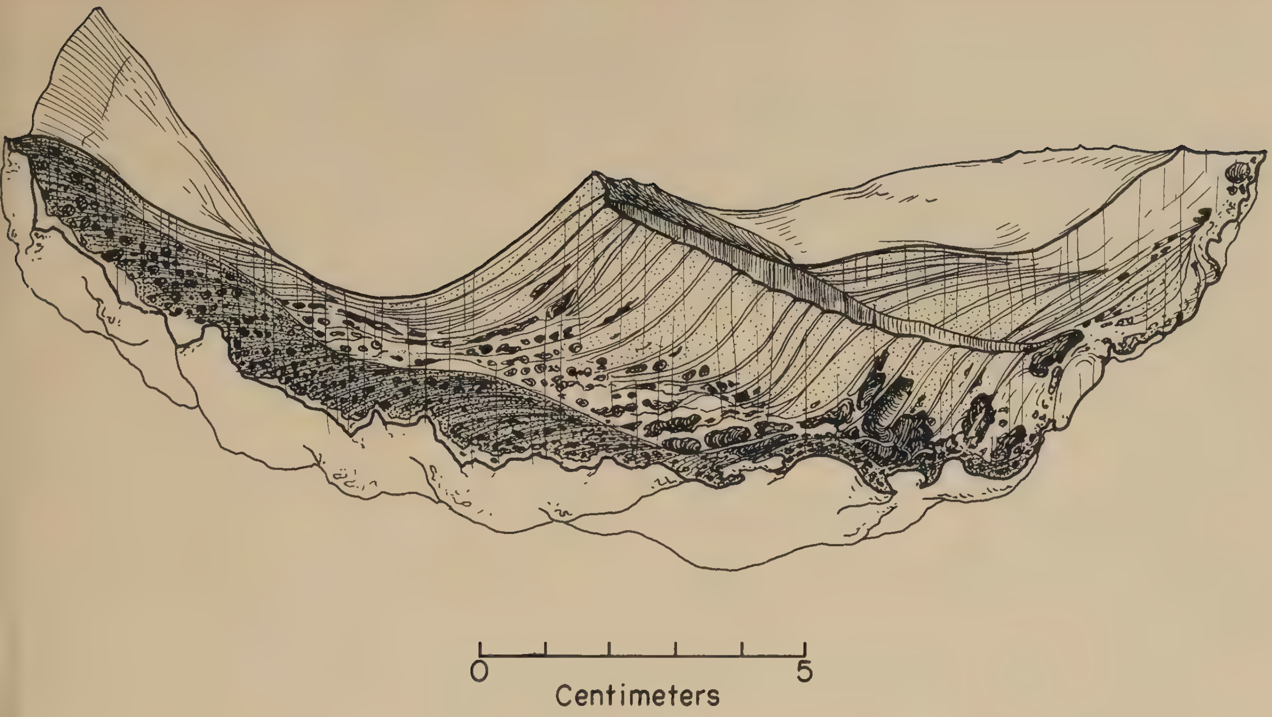

FIGURE 48.—Left valve of *O.* (*Alectryonia*) *megodon* cut along the principal axis of growth. Hypostracum (dark striated layer) forms a pronounced platform for the attachment of the adductor muscle, and can be traced to its original position in the young oyster (right). Chalky deposits are regularly arranged between the layers of calcite. Also see fig. 41.

cribed for calcite shells. Present knowledge of he chemistry of the organic constituents of the hell is inadequate. It seems reasonable to ssume that conchiolin like other proteins is not single chemical substance common to a large umber of organisms, but that it differs specifi- ally from animal to animal and may even vary n the different parts of the same shell.

The analysis of amino acids obtained by hy- rolysis of conchiolin prepared from decalcified hells showed (Roche, Ranson, and Eysseric- afon, 1951) that there is a difference in the shells f the two species of European oysters, *O. edulis* nd *C. angulata* (table 4).

TABLE 4.—*Amino acids from the conchiolin of two species of oysters*

[In parts of 100 parts of protein according to Roche, Ranson, and Eysseric-Lafon (1951)]

Amino acids	*Crassostrea angulata*	*Ostrea edulis*
Arginine	0.45	2.90
Histidine	----------	0.65
Lysine	3.55	4.30
Glycine	15.70	15.70
Leucine	0.51	----------
Tryptophane	----------	0.48
Tyrosine	3.27	3.05
Valine	0.95	----------
Cystine	----------	0.98
Methionine	1.77	1.62

Taking advantage of the fact that both calcite and aragonite are present in the two distinct layers of shell of the fan oyster (*Pinna*) and of the pearl oyster (*Pinctada*), the French investigators (Roche, Ranson, and Eysseric-Lafon, 1951) attempted to determine whether there is a difference in the chemical composition of the organic material of the two layers of the shell of the same species. They found that tyrosine and glycine occur in higher concentrations in the prismatic layer than in the nacreous part of shells. In the prismatic layer of calcite portion the content of tyrosine varies between 11.6 and 17.0 percent and that of glycine between 25 and 36 percent. In the nacreous part made of aragonite the concentration of tyrosine was from 2.8 to 6.0 percent and that of glycine varied between 14.9 and 20.8 percent. The significant differences in the contents of the two amino acids in the two parts of the shell may provide a clue for further studies of the role of the organic component on the mineral form in which the calcium carbonate is deposited by the mantle.

MUSCLE ATTACHMENT

The place of attachment of the adductor muscle or muscle scar is the most conspicuous area of the

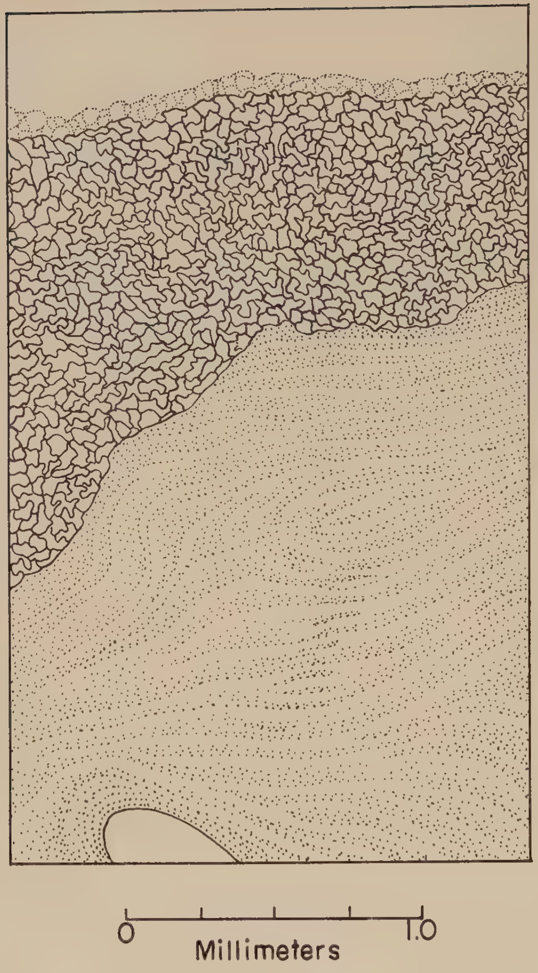

FIGURE 49.—Cross section of shell of an adult *C. virginica* after decalcification in weak acid, Mallory triple stain. Conchiolin of the prismatic layer is reddish-brown; that of calcite-ostracum is bluish.

oyster shell. In *C. virginica*, *C. angulata*, and many other species this area is highly pigmented; in *O. edulis*, *C. gigas*, pigmentation is either absent or very light.

The muscle scar in *C. virginica* is located in the posteroventral quadrant of the shell (figs. 15, 21, 33). To a certain extent the shape of the scar reflects the shape of the shell, being almost round in broad and round oysters and elongated in narrow and long shells. The area of scar is slightly concave on the side facing the hinge and convex on the opposite, i.e., ventral side. Curved growth line, parallel to the curvature of the ventral edge of the valve, can be seen on the surface. They are most pronounced in the ventral part of the muscle impression. Size and shape of the scar is variable and often irregular (fig. 50). The outlines of the impressions shown in this figure were obtained in the following manner: the periphery of the impression was circumscribed with soft pencil; a piece of transparent Scotch adhesive tape was pressed on the impression and the outline was lifted and mounted on cross-section paper; the area occupied by the impression was measured by counting the number of squares. Using this method, I obtained the replicas of muscle impressions from 169 shells taken at random from various oyster beds of the Atlantic and Gulf Coasts. The impressions are arbitrarily arranged in four series (A–D) according to their shape and size. The impression areas of round and broad shells are shown in the two upper rows, A and B; those of long and narrow shells are arranged in the two lower rows, C and D.

It may be expected that the larger is the shell the greater is the area of muscle impression. The relationship, as can be seen in fig. 51, is rectilinear although the scatter of plotted data is considerable and the variability increases with the increase in size. The ratio of muscle impression area to shell surface area varies from 8 to 32 with the peak of frequency distribution at 16 to 18 (fig. 52).

A small oval and unpigmented area on the

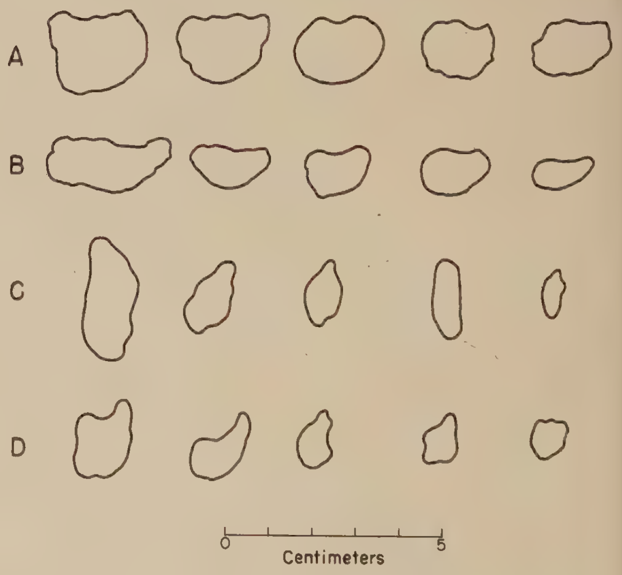

FIGURE 50.—Variations in shape and size of muscle scars on the shells of *C. virginica*. Rows A and B show the types of scars normally found on broad and rounded shells, the length of which is almost equal to or exceeds the height. Rows C and D are the scars often found on long and narrow shells in which the height exceeds the length. Replicas of scars were made from shells collected at random.

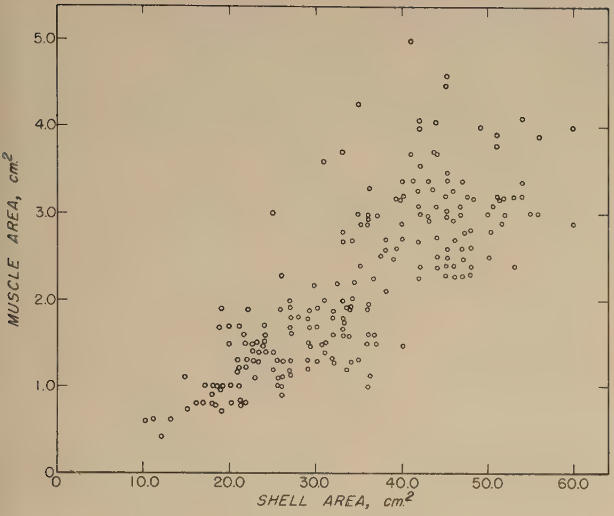

FIGURE 51.—The relationship between the area of muscle scar and the area of the shell of *C. virginica*.

dorsal half of each valve is the imprint of a vestigial muscle in the mantle, discovered in 1867 by Quenstedt in the valves of the early Jurassic oyster, *Gryphaea arcuata* Lamarck, and found by Stenzel (1963) in *C. virginica*. In my collection of living *C. virginica* the imprint is hardly visible (figs. 15, 21, and 22). Slight adhesion of the mantle to the valve indicates the location of this area which Stenzel calls "imprint of Quenstedt's muscle."

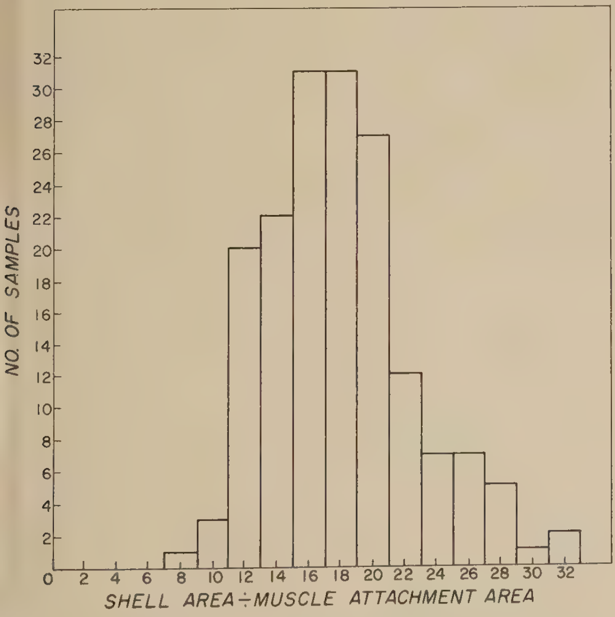

FIGURE 52.—Frequency distribution of the ratio of muscle scar area vs. shell area in the shells of *C. virginica* of Atlantic and Gulf States.

TABLE 5.—*Chemical composition of oyster shells in percent of shell weight*

[From Hunter and Harrison, 1928]

Constituents	Sample 1	Sample 2
Al	0.045	0.043
Ca	38.78	38.81
Cu	----------	0.0025
Fe	0.11	0.09
Mg	0.183	0.189
Mn	0.009	0.009
P_2O_5	0.075	0.073
SiO_2	0.570	0.580
Zn	----------	0.0009
Cl	0.0034	0.0035
CO_3	[3] 57.19	----------
Fl	----------	----------
N	0.196	0.196
As	----------	----------
Organic matter [1]	1.41	1.51
Water [2]	0.27	0.28

[1] Loss above 110° C. Ignited.
[2] Loss to 100° C.
[3] Average for samples 1 and 2.

CHEMICAL COMPOSITION

The oyster shell consists primarily of calcium carbonate, which composes more than 95 percent of the total weight of the shell. The balance is made up by magnesium carbonate, calcium sulfate, silica, salts of manganese, iron, aluminum, traces of heavy metals, and organic matter. Several analyses of oyster shell found in the literature are incomplete, particularly with reference to trace elements. Analysis made for the U.S. Bureau of Fisheries by the Bureau of Chemistry of the Department of Agriculture and published in 1928 (Hunter and Harrison, 1928) is given in table 5.

Dead oyster shells buried in the mud of the inshore waters of Texas and Louisiana are extensively dredged by commercial concerns primarily for the manufacture of chicken feed. Analysis of these shells as they are received at the plant after thorough washing in sea water is given in table 6.

The calcium carbonate content of these shells is probably lower than in live oysters due to their erosion and dissolution of lime in sea water. The chloride content is affected by the retention of

TABLE 6.—*Chemical composition of mud shells received at the plant of Columbia-Southern Corporation at Corpus Christi, Tex.*

[Percent of constituents in samples dried at 110° C.]

Chemical	Percent
$CaCO_3$	93.88
SO_4 as $CaSO_4$	0.48
$MgCO_3$	0.88
SiO_2	1.40
$Al_2O_3+Fe_2O_3$	0.32
Cl as NaCl	0.27
Na_2O (other than NaCl)	0.46
Loss at 500° C	1.60

(Analysis supplied by Columbia-Southern Corporation and copied with their permission.)

these salts in the shells after thorough washing with sea water of greatly variable salinity. The percent of silica, aluminum, and iron, which are also higher than in the analyses of shells of live oysters, is at least in part influenced by the efficiency of plant operations in removing mud from the surface of the shells.

Chemical composition of shells of *O. edulis* is not significantly different from that of *C. virginica.* Table 8 gives the results obtained by European scientists. The data quoted from various sources are taken from Vinogradov (1937).

A much more detailed analysis of dead oyster shells dredged from the bottom of Galveston Bay 8 miles east of San Leon was made recently by the Dow Chemical Company (Smith and Wright, 1962). The shells were scrubbed in tap water with a nylon brush, rinsed in distilled water, dried at 110° C., and ground in a porcelain mortar. With the kind permission of the authors the results are given in table 7. Additional 19 elements were sought but not found at the following sensitivity limits:

10 p.p.m.—arsenic, barium.
1 p.p.m.—antimony, chromium, cobalt, germanium, gold, lead, lithium, mercury, molybdenum, nickel, vanadium, and zirconium.
0.1 p.p.m.—beryllium, bismuth, cadmium, silver, and tin.

The authors remark that traces of clay entrapped within the shell may have influenced the findings for titanium, manganese, copper, or zinc; and that individual variations in silicon, iron, and aluminum were due to contamination not removable by washing. It appears feasible that these variations may have been caused by spicules of boring sponges and algae infesting the shells.

TABLE 7.—*Composition of* C. virginica *oyster shell dredged from Galveston Bay, according to Smith and Wright (1962)*

Constituent	Concentration	Constituent	Concentration
	Percent		*P.p.m.*
Calcium (CaO)	54.6	Organic Carbon as CH_4	400
Carbon (CO_2)	43.5	Chlorine (Cl)	340
Sodium (Na_2O)	0.32	Aluminum (Al)	200
Magnesium (MgO)	0.33	Iron (Fe)	180
Sulfur (SO_3)	0.16	Phosphorus (P)	116
Silicon (SiO_2)	0.16	Manganese (Mn)	110
Strontium (SrO)	0.12	Fluorine (F)	54
Moisture (H_2O)	0.58	Potassium (K)	30
		Titanium (Ti)	12
		Boron (B)	5
Total of major constituents	99.8 %	Copper (Cu)	3
		Zinc (Zn)	2
		Bromine (Br)	1
		Iodine (I)	0.5

TABLE 8.—*Chemical composition of shells of* O. edulis *(in percent of ash residue)*

Constituent	Sample 1	Sample 2	Sample 3	Sample 4
$CaCO_3$	98.60	97.65	96.54	97.00
$Ca_3(PO_4)_2$	1.21	----------	----------	----------
$MgCO_3$	----------	0.312	0.9125	trace
P_2O_5	----------	0.52	0.058	0.09
$(Al, Fe)_2O_3$	----------	trace	----------	0.03
Fe_2O_3	0.0719	----------	----------	----------
$CaSO_4$	----------	1.456	----------	2.00
SiO_2	0.813	----------	----------	----------
Organic matter	----------	0.50	0.5–4.5	----------

According to Creac'h (1957), all shells of *O. edulis* and *C. angulata* contain traces of phosphorus. The French biologist found that the phosphorus content is variable. Expressed as P_2O_5, it varies in *C. angulata* from 0.075 to 0.114 percent. There is a significant difference in the phosphorus content in various parts of the shell. The amount of phosphorus per unit of volume of shell material is lower in the chalky deposits than in the hard portion of the shells. Thus, in laying a chalky deposit the mollusk utilizes from 2.4 to 2.6 times less phosphorus than is needed for secreting the same volume of harder shell substance.

The presence of small quantities of strontium in calcareous shells of mollusks is of particular interest because of its apparent relation to aragonite. The marine organisms containing calcium carbonate as aragonite have relatively higher strontium content than those having calcite shells. The relationship between the two elements is expressed as strontium-calcium atom ratios (Thompson and Chow, 1955; Trueman, 1944; and Asari, 1950). In *C. virginica* and *C. gigas* the strontium-calcite ratio x 1,000 varies between 1.25 and 1.29. *Ostrea lurida* from California has a lower strontium content, the ratio being 1.01. The percentages of Ca, Sr, CO_2, and organic matter in the shells of three species of oyster and in *Mya arenaria*, in which the content is the highest among the bivalves, given by Thompson and Chow (1955), are summarized in table 9. The

TABLE 9.—*The percentage of calcium and strontium in the shells of oysters and soft shell clam*

[According to Thompson and Chow, 1955]

Species	Calcium	Strontium	Carbon dioxide	Organic matter	Atom ratio Sr/Ca x1,000
O. lurida	38.6	0.085	42.5	1.68	1.01
C. virginica	33.7–37.8	0.92–0.107	41.8–42.4	2.16–2.34	1.25–1.29
C. gigas	34.6–36.2	0.097–0.100	32.6–42.5	1.33–1.71	1.26–1.28
M. arenaria	38.6–38.8	0.181–0.246	42.2–42.3	2.22–2.44	2.16–2.91

salinity and temperature of the water have apparently no influence on Sr/Ca, which remains fairly constant in calcareous shells. The possible role of strontium in the mineralization and formation of shell is discussed in chapter V.

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CHAPTER III

THE LIGAMENT

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APPEARANCE AND STRUCTURE

The significance of the ligament in the phylogeny and classification of bivalves was a favored subject in malacological studies of the past century. Lengthy theoretical speculations about this structure are found in the papers of Bowerbank (1844), Jackson (1890, 1891), Tullberg (1881), Dall (1889, 1895), Reis (1902), Biedermann (1902), Stempell (1900), and others. A review of the literature from the earlier years to 1929 is adequately presented by Haas (1935). These investigations give little information, however, concerning the microscopic structure, origin, chemical composition, and function of the ligament. The latter subjects receive attention in the more recent works of Mitchell (1935) on the ligament of *Cardium corbis*, in a series of detailed studies by Trueman (1942, 1949, 1950a, 1950b, 1951, 1952, 1953a, 1953b) on the ligaments of *Mytilus*, *Pecten*, *Nucula*, *Ostrea edulis*, *Tellina tenuis*, and the Semelidae, and in the paper of Owen, Trueman, and Yonge (1953) on the ligament in the bivalves.

The ligament of the Atlantic oyster is a narrow band of dark, elastic material situated along the edge of the hinge between the two valves. The ligament does not extend deep into the shell, is not visible from the outside, and is called internal or ligamentum internum by Haas (1935) and "alvincular" by Dall (1889). The latter term is no longer used in malacological literature.

The ligament performs a purely mechanical function. Its elastic material, compressed when the contraction of the adductor muscle closes the valves, expands and pushes the valves apart when the tension of the adductor is released. The extent to which the valves may gape depends largely on the shape and size of the beaks. In the specimen shown in figure 17 the large, triangular space beyond the hinge permits wide excursions of the valves and their gaping may consequently be very broad.

On the other hand, the narrow and crooked beaks shown in figure 53 greatly restrict the movement of the valves along the pivotal axis regardless of the degree of relaxation of the muscle. Small pebbles, pieces of broken shell, and other foreign particles often found lodged between the beaks may further limit the opening of the valves. The possibility that such purely mechanical obstructions can impede the movement of the valves should be kept in mind in evaluating the results of physiological tests in which the degree of shell opening is recorded.

The youngest part of the ligament is that which touches the inside of the valves; the oldest portion, which is usually dried, cracked, and nonfunctional, faces the outside. When the

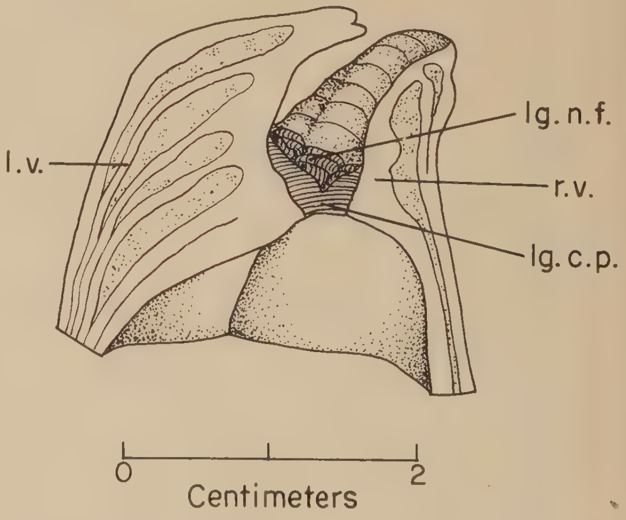

FIGURE 53.—Longitudinal section through the beak and ligament of *C. virginica*. l.v.—left valve; r.v.—right valve; lg.c.p.—functional, compressible part of the ligament; lg.n.f.—nonfunctional, old part of the ligament.

valves are forcibly separated, the ligament breaks approximately along the pivotal axis of the shell (fig. 54, piv. ax.) and the two parts remain attached to the respective valves.

The three parts of the ligament at the edge of the valves differ in color, size, and shape. The usually brownish central (inner) part called resilium forms a bulging ridge marked by fine striations visible to the naked eye or under a low magnification. The resilium is attached to a groove called resilifer or chondrophore (figs. 54, 2, 16). The dark olive anterior and posterior portions of the ligament called by Olsson (1961) tensilia are attached to the edges of the valves (nymphae).

The resilium consists of tightly packed lamellae arranged at about right angles to the longitudinal axis of the ligament; they can be seen on the exposed surface of the central part. These lamellae are intersected by fine striations visible on the side of the resilium after the removal of the adjacent lateral part (fig. 55).

When the valves are closed the resilium is compressed because of its considerable thickness while both lateral parts (tensilia) are slightly stretched. It can be seen in a series of cross sections of the hinge made at right angles to its pivotal axis (fig. 56) that the curved lines of the compressed resilium (2) are deeply arched, while those in the lateral parts are almost straight. This observation agrees with the description of the operation of the ligament of *O. edulis* by Trueman (1951). Since the beaks of the oyster illustrated are asymmetrical, the distance between the two valves is greater at the anterior than at the posterior end (fig. 56, 3, and 1) and, consequently, the anterior portion of the ligament stretches more than its posterior part.

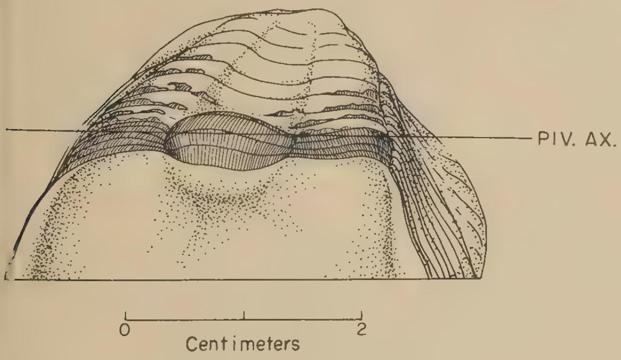

FIGURE 54.—Ligament of large *C. virginica* attached to the right valve. View from the inside. piv. ax.—pivotal axis. The resilium occupies the central position and on both sides is flanked by tensilium. Slightly magnified.

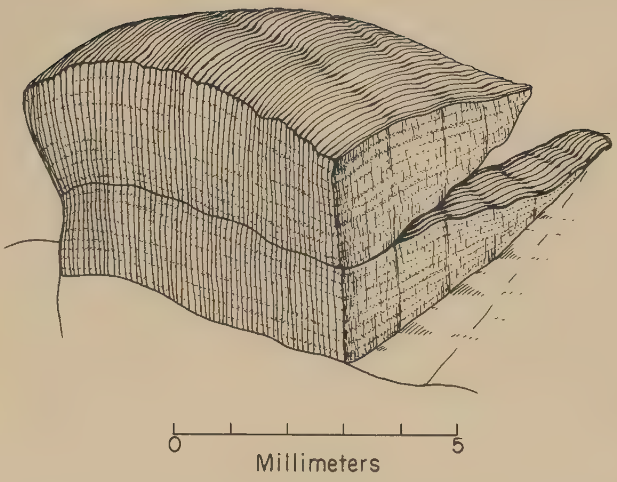

FIGURE 55.—Central portion of the ligament (resilium) attached to the groove (bottom) of a valve. Note the lamellar structure and fine striations visible on the right side of the figure. *C. virginica*.

The ligament effectively seals the space between the dorsal edges of the valves and forms an elastic, watertight joint that prevents the entry of water and organisms which otherwise could easily invade the mantle cavity.

The spring-like action of the ligament is a function of the elasticity of its component parts. Examination of transverse and longitudinal sections of fresh ligament under low power discloses its amazingly complex structure. A cross section made with a razor blade at a right angle to the pivotal axis of the valves shows a series of well-defined curved lines extending from the right to the left valve, and a complex system of lamellae arranged perpendicularly to the curves. Both systems are clearly seen in unstained preparations mounted in glycerin jelly or in balsam (fig. 57). The pivotal axis of the ligament lies in the center of the drawing, perpendicular to the plane of the paper; the valves (not shown in the figure) are on the right and left sides, and the newly deposited portion of the ligament lies at the bottom of the drawing. The most conspicuous arches extend almost without interruption from one side to another; the lighter ones can be traced only for short distances over the cross-sectional area. The structure of the resilium resembles a leaf plate of an old-fashioned automobile spring, suggesting that the arches are the lines of stresses corresponding to the deformation of the ligament under compression. Within the mass of the ligamental ma-

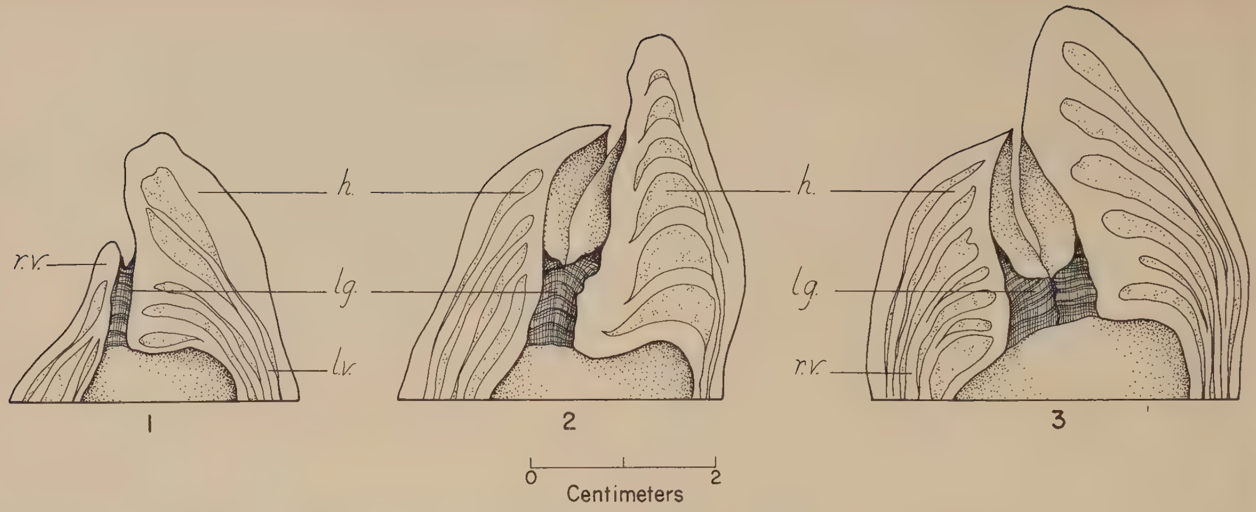

FIGURE 56.—Three longitudinal sections through hinge and ligament of the shell of *C. virginica*. (1) posterior portion; (2) central portion or resilium; (3) anterior portion, h.—beaks, lg.—ligament, l.v.—left valve, r.v.—right valve. Note the arched lines of the resilium (lg.) in the central drawing (2). Slightly magnified.

terial they are the visual evidence of these stresses. Since the "springs" of the resilium do not consist of separate structure parts joined together into a complex unit, the comparison is only superficial.

The ligament is a nonliving structure secreted at a varying rate by the highly specialized epithelium of the subligamental ridge of the mantle (see p. 89). Structurally, the arches, visible at low magnification, represent stages of growth; functionally and in accentuated form, they reflect compressional deformation in the operating structure of the ligament.

Under a binocular microscope the lamellae of the resilium, when separated with fine needles, appear slightly bent and zigzagged. A small piece of the resilium cut in the dorsoventral plane and magnified about 250 times (fig. 58) can be seen to consist of fibrillar material and of dark bands of variable width composed of tightly packed, oval, birefringent globules. Pressure over the cover slip does not change the shape of the globules, which appear to be firmly embedded in the ground substance. The globules contain no acid-soluble material since they are not affected by strong hydrochloric acid, nor are they soluble in alcohol or xylol. Preparations mounted in balsam present the same appearance as nondehydrated sections mounted in glycerin jelly. Besides the globules concentrated in the dark bands within a delicately fibrillar ground substance, some of them are arranged in longitudinal lines at right angles to the dark bands. Some of the horizontal bands (upper part of figure 58) are of much greater complexity than the others; they consist of oval-shaped light areas surrounded by globules. The two structural elements, namely, the bands of fibrils and the rows of globules, repeat themselves with regularity, the successive layers varying only in width and in the concentration and size of globules. The fibrils intersect the arches either perpendicularly or at about 45° (lower part of figure 58) and probably exert additional elastic force under compression.

The anterior and posterior parts of the ligament, the tensilium of Olsson (1961) or outer layer of Trueman (1951), are made of tenacious material which withstands considerable stretching without breaking. This can be easily ascertained by trying to tease or to pull apart the dissected parts of the tensilium. In this respect the material of the tensilium differs from that of the resilium, which is weak under tension but strong under compression. The color of the tensilium differs from that of the resilium. In New England oysters it is usually dark green on the surface, while the resilium is light brown. The tensilium is made of tough lamellae which in a transverse section appear as slender, transparent cylinders of slightly yellowish substance (fig. 59). Both resilium and tensilia are secreted by highly specialized epithelial cells which underly the ligament. The thickness of each lamella corresponds to the width of a ruffle at the edge of the secreting epithelium (See chapter V, p. 89). At low magnification the material of the tensilium appears to be non-

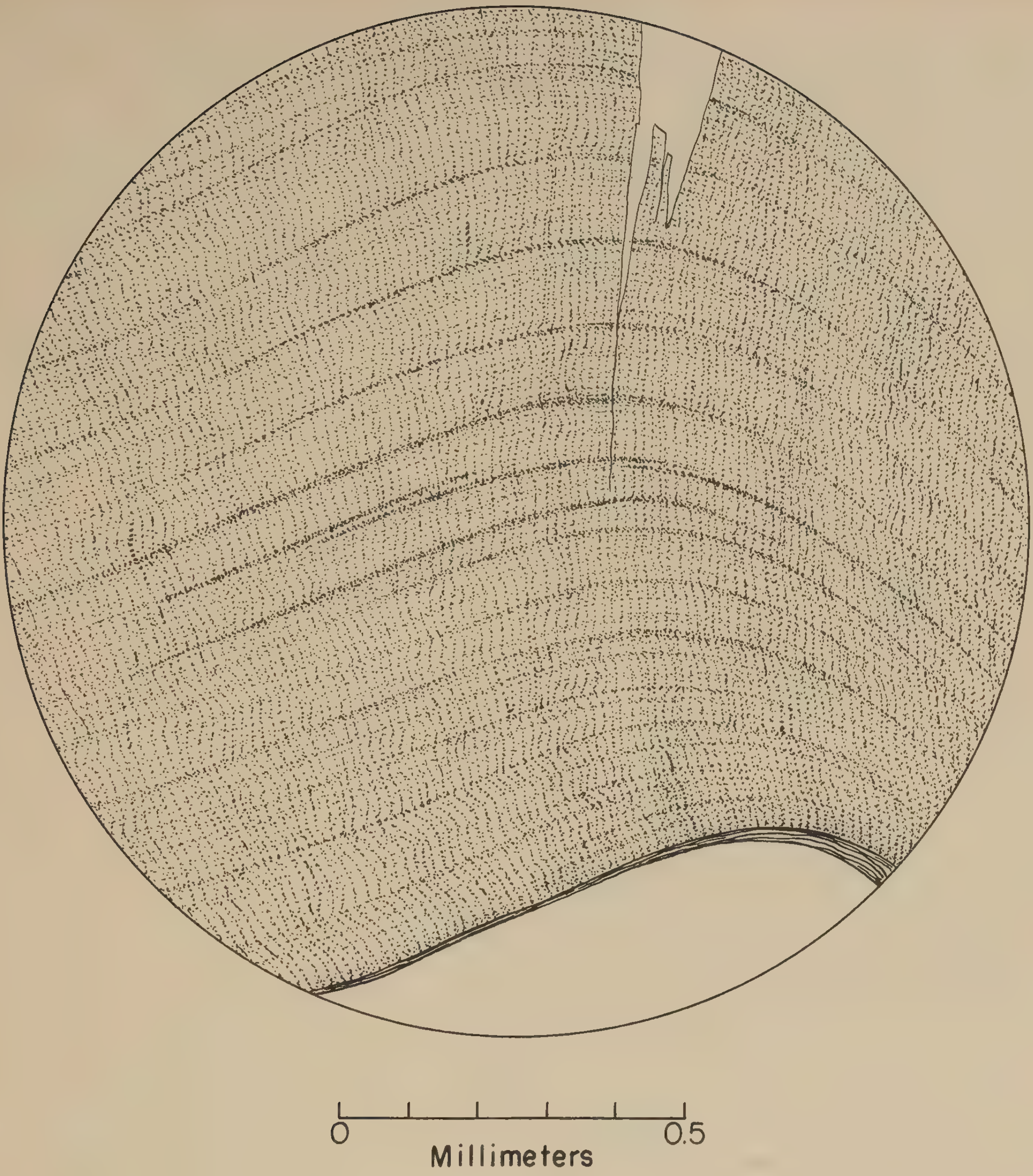

Figure 57.—Cross section of the central portion of the ligament of *C. virginica* made perpendicular to the pivotal axis of the valve. Arches (curved lines of dense material) extend from left to right; the valves are not shown in the drawing.

fibrillar, but at higher magnification the fibrillar structure becomes clearly visible. Two types of fibrils can be distinguished on the photomicrograph of tensilium shown in figure 60. Heavy and well-defined bundles of fibers originated along the vertical plane of the lamellae (up and down bundles in fig. 60) and short and slender fibrils in places at right angles to the large bundles (the lower half of fig. 60). Large oval-shaped bodies on the upper right and lower left part of the figure are the accumulation of calcium carbonate crystals. Single minute crystals are scattered over the body of the lamella. The outer dark layer is very thin, its color is due to densely packed narrow fibrils. Large and small globules which are conspicuous in the architecture of the resilium are absent in the tensilium, and the structure of the latter lacks the complex arrangement of globules and fibrils found in the former.

The complexity of the microscopic structure

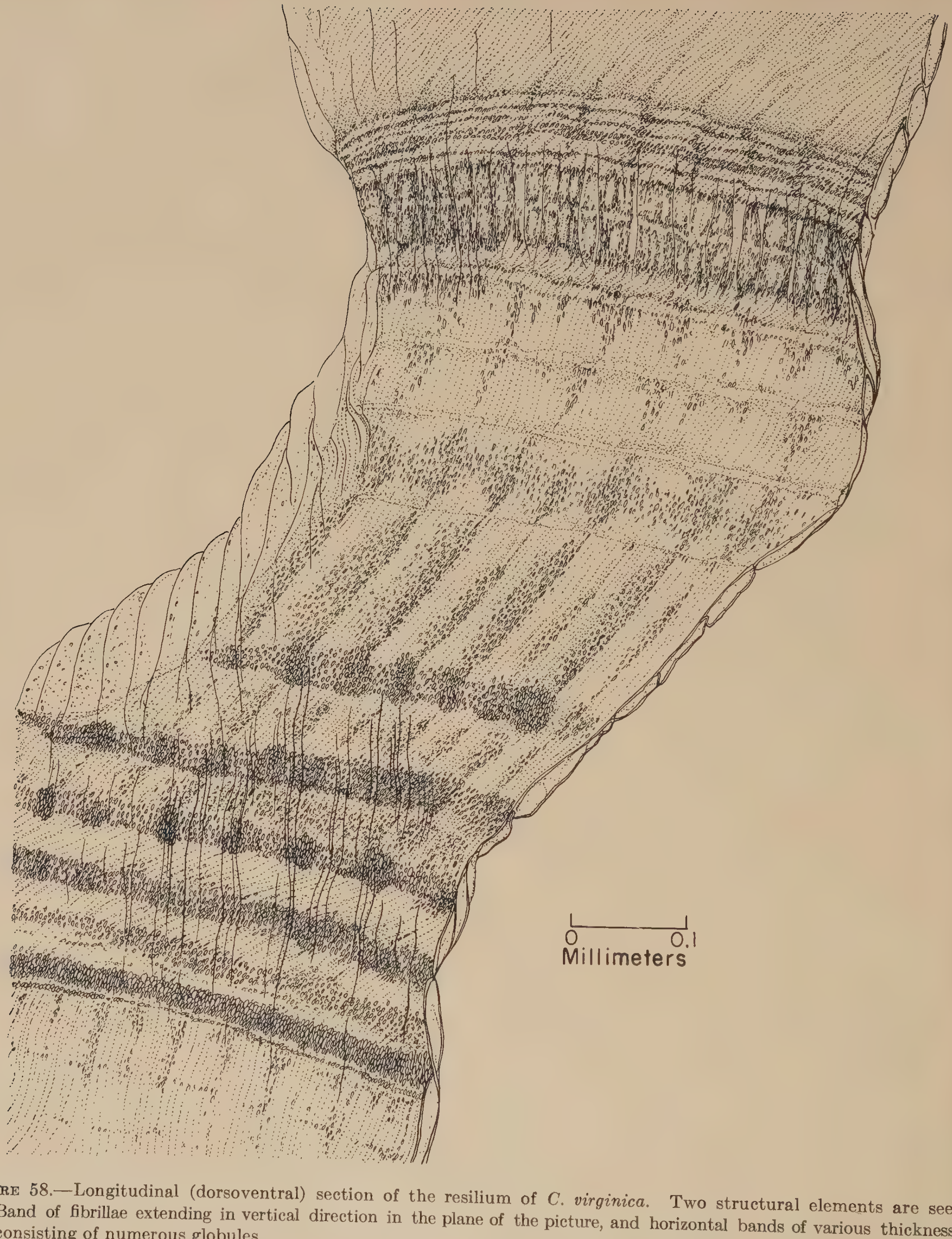

FIGURE 58.—Longitudinal (dorsoventral) section of the resilium of *C. virginica.* Two structural elements are seen: Band of fibrillae extending in vertical direction in the plane of the picture, and horizontal bands of various thicknesses consisting of numerous globules.

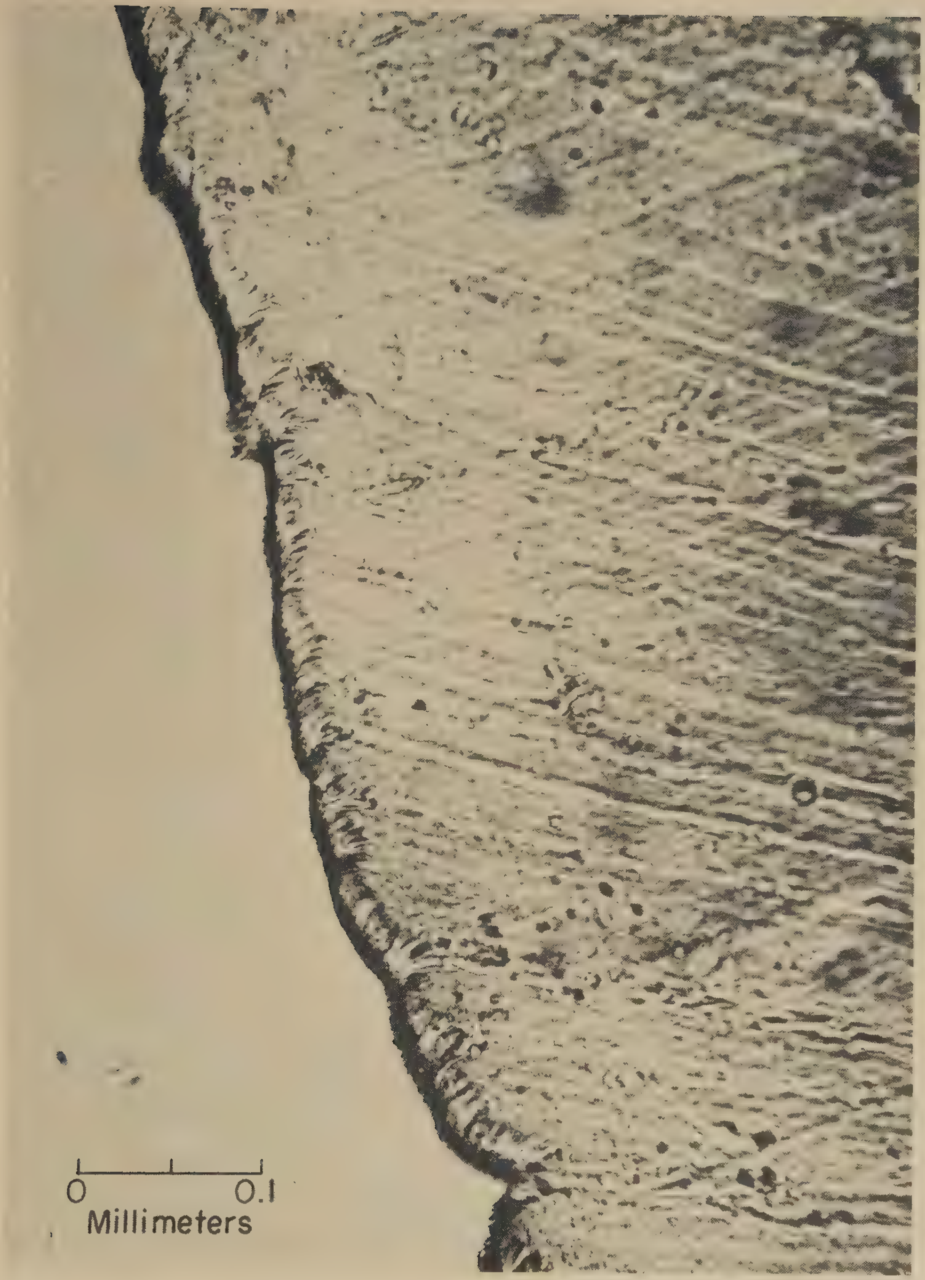

FIGURE 59.—Transverse section of tensilium showing lamellar structure and darkly pigmented surface, *C. virginica*. Photomicrograph of unstained and nondecalcified preparation.

suggested that electron microscopy might reveal some interesting details. Small pieces of the resilium fixed in 1 percent osmic acid were embedded in plastic and sectioned. Although the material is very hard, it was possible to obtain sections from 0.3 to 0.5μ in thickness. The electron micrograph (fig. 61) shows bands of fibrils varying in diameter from 370 to 500 Å.

A section made across the plane of the arches (fig. 62) shows a membrane honeycombed by holes about 500 Å in diameter. Two interpretations seem possible: (1) that the fibrils are tubular, the light areas corresponding to the centers of the tubes, or (2) that the empty circles represent spaces between the fibrils. The first interpretation is more plausible because of the gradation

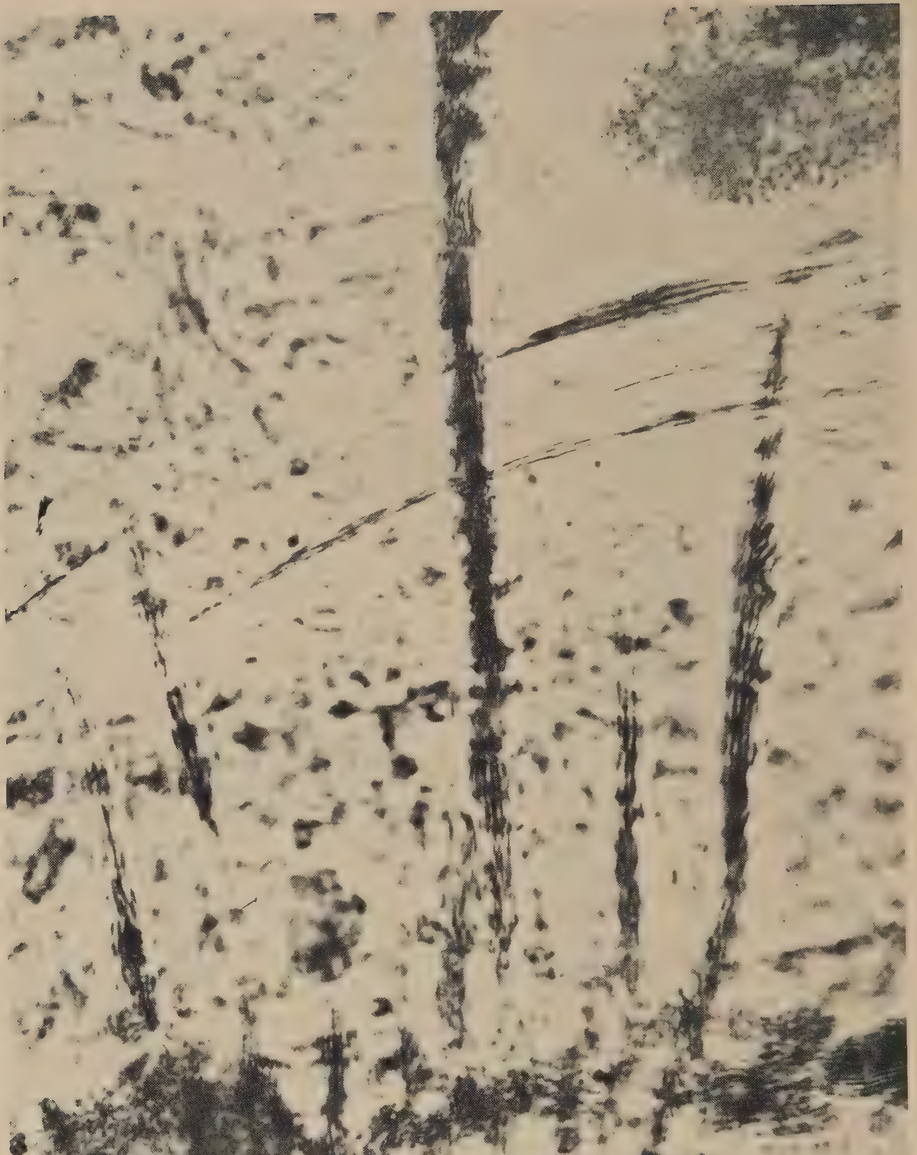

FIGURE 60.—Large and small bundles of fibers of the tensilium of *C. virginica* seen in unstained and nondecalcified preparation of the material teased in glycerin. Photomicrograph.

from circular to elliptical light areas as the plane of section of the fibrils becomes tangential (see fig. 62).

Sections made at right angles to the fibrils (fig. 62) demonstrate a certain similarity to those of the organic membranes of the aragonitic part of the shells of mollusks and pearls. According to Grégoire, Duchâteau, and Florkin (1950, 1955), such organic membranes have a lace-like structure consisting of meshes and holes of different size and pattern. In these investigations by Belgian biologists the material was first decalcified, and the layers of organic substance then separated by ultrasonic oscillation to obtain the ultrathin membranes suitable for electron microscopy. The films of the calcite-ostracum layer of the shells of

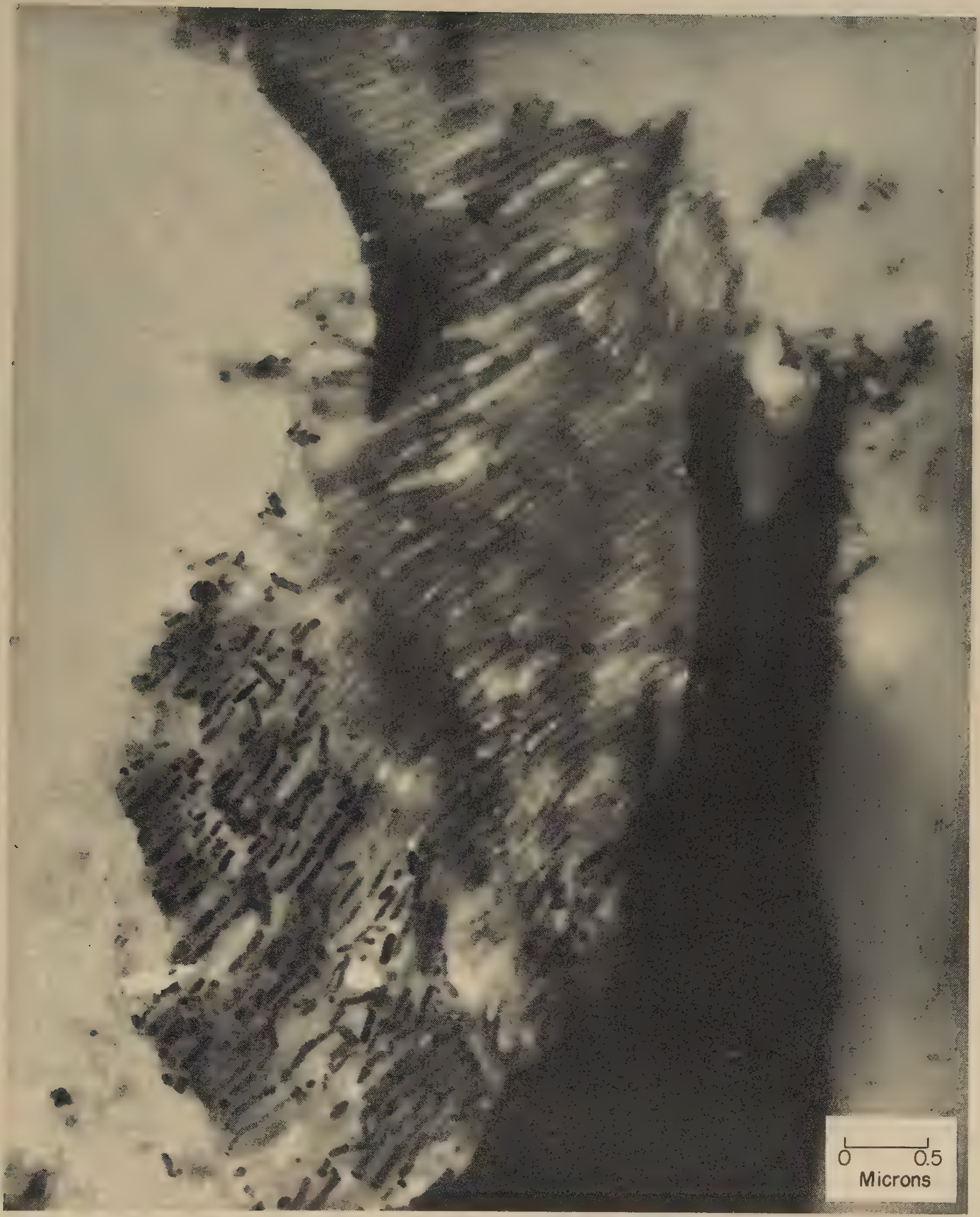

FIGURE 61.—Electron micrograph of the ligament of *C. virginica* sectioned parallel to the fibrils.

pelecypods which have no true nacre (*O. edulis*, *O. tulipa*, *Yoldia*, *Acra*, and others) were found to consist "of heterogenous material, the more representative elements of which are amorphous, vitreous plates, sometimes granular and devoid of visible (or unquestionable) pores." (1950, p. 30).[5] In the absence of ultrasonic equipment in my laboratory this method could not be used at Woods Hole, Mass. Comparison of figures published by Grégoire and his associates with the photograph reproduced in figure 62 suggests that

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[5] Translation by Paul S. Galtsoff.

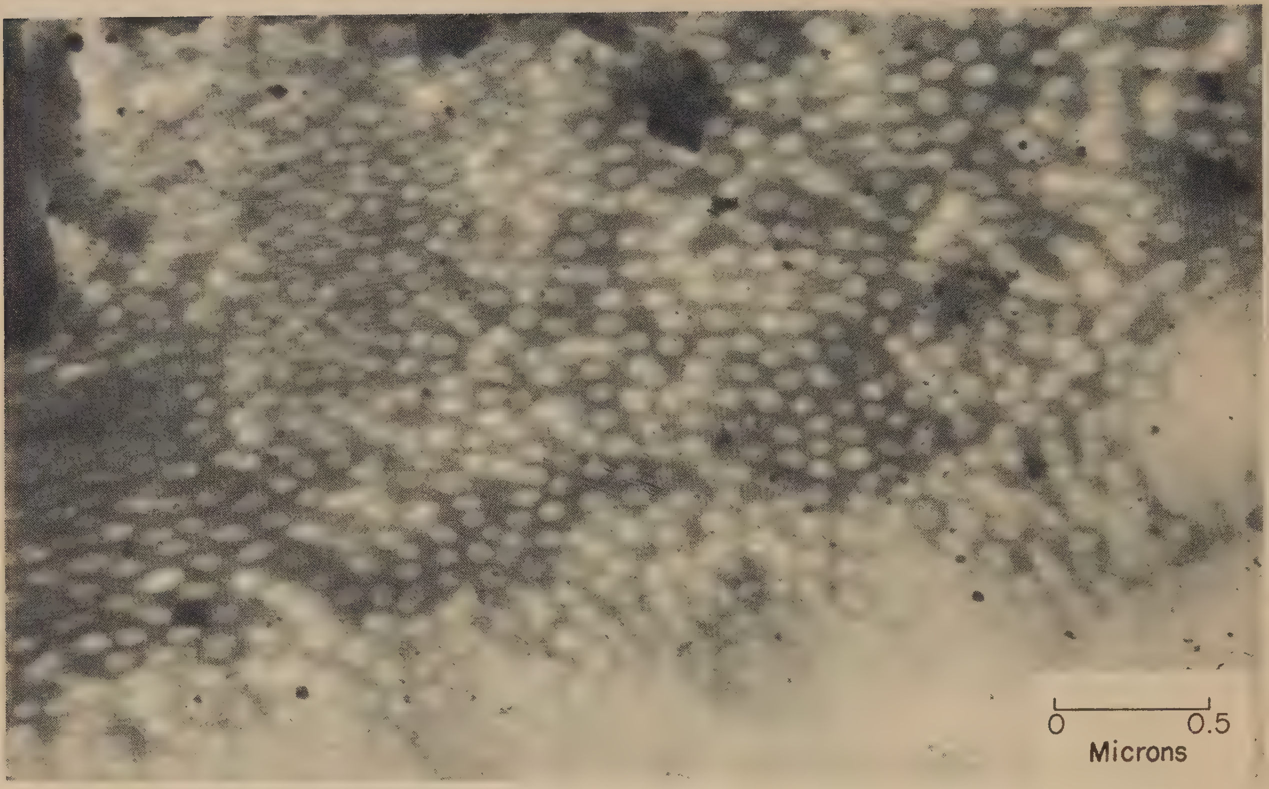


FIGURE 62.—Electron micrograph of a section of the ligament of *C. virginica* made across the fibrils.

the structure of the ligament of *C. virginica* has some similarity to that of the organic membranes of the aragonite shells. Recently Stenzel (1962) has found that the resilium of the Ostreidae contains aragonite.

One of the sections of the ligament of *C. virginica* studied with the electron microscope shows a series of black, oval-shaped bodies arranged along curved lines and separated from one another by fibrils (fig. 63). The black bodies probably correspond to the small globules visible under the light microscope. Their nature has not been determined.

The action of the ligament can be demonstrated by a rather crude model consisting of two slightly curved pieces of wood, representing the valves, joined by a series of brass rods. The rods are bent and arranged to correspond to the course of the arches as the latter are seen in an enlarged photograph of a transverse section of the ligament (fig. 57). Thin rubber tubing interwoven between the arches corresponds to the bundles of fibrils. Since the diameter of rubber tubing used in the construction of the model greatly exceeds the comparable diameter of the fibrils, this portion of the model is not in scale. Another departure from actual conditions is the interweaving of the rubber tubing between the arches, a method used to simplify construction although no such arrangement of fibrils was disclosed by microscopy. The model is shown in fig. 64. If the sides of the structure are pressed together, the arches curve up and exert lateral pressure at the same time that the increased rigidity of the rubber tubing adds to the elastic force. One can easily feel this pressure by touching the rubber tubing with the finger tips while bringing the "valves" together.

CHEMICAL COMPOSITION

The chemical composition of the ligament is essentially the same as that of the organic matrix of the shell (Mitchell, 1935: Trueman, 1949, 1951). The proteins forming the lateral (tensilium) and the central (resilium) portions of the ligament are not, however, identical. The difference can be demonstrated by staining reactions and by various chemical tests. For instance, in *Tellina tenuis* the lateral parts of the ligament are stained red or yellow by Mallory triple stain, while the inner part turns blue, a difference comparable to that between the staining reaction

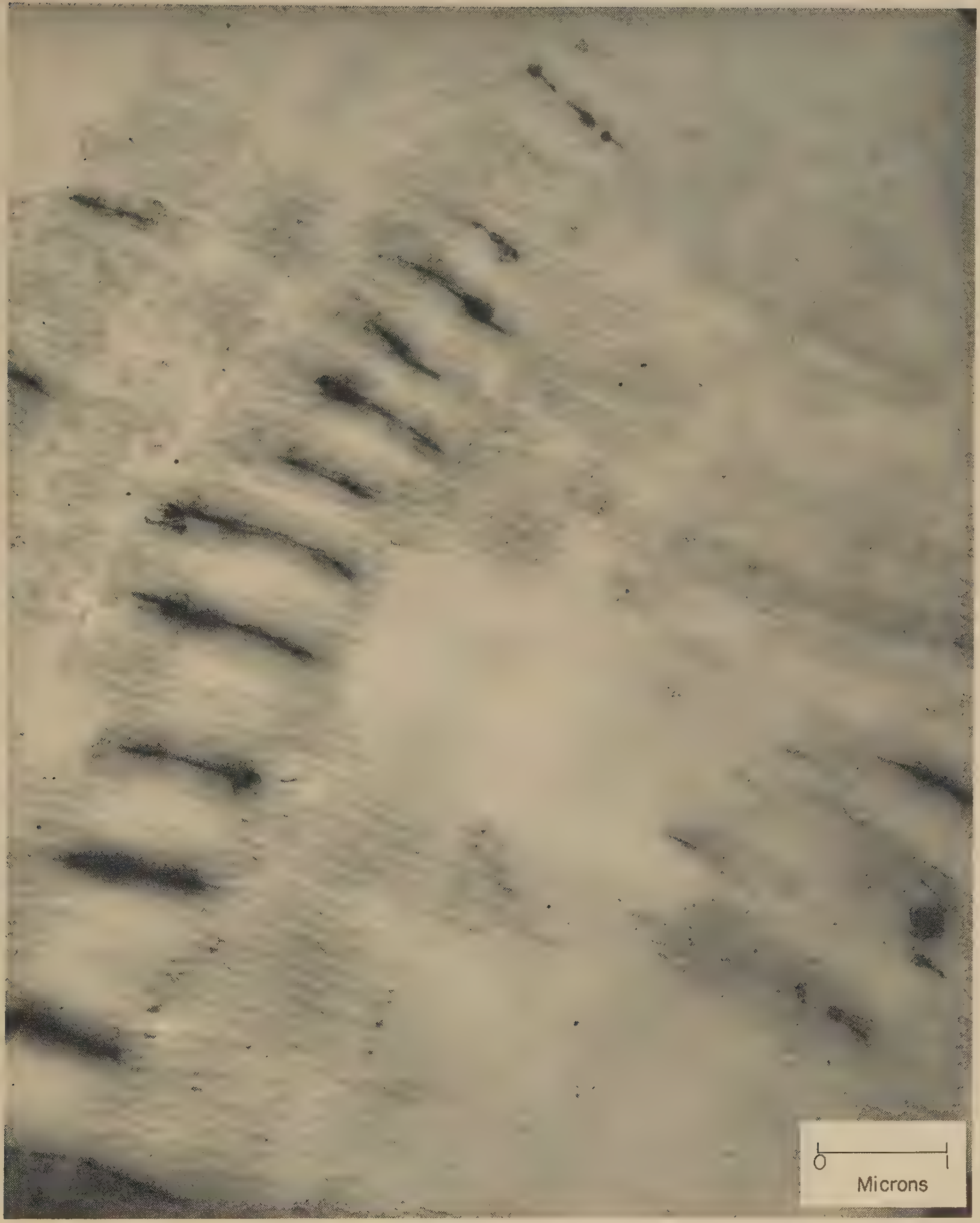


Figure 63.—Electron micrograph of the ligament made near one of the arches parallel to the fibrils of *C. virginica*. Dark bodies probably correspond to the smallest globules seen in the light microscope.

of the conchiolin of the prismatic layer and that of the calcite-ostracum discussed on p. 42. Trueman (1949) concludes that the two types of conchiolin seem to correspond respectively to the two components of the ligament. The tensilium gives a positive reaction with the xanthoproteic, Millon's, and Merker's reagents, whereas the reaction of the resilium to these reagents is negative. Brown (1949) points out that most of the epithelial skeletal proteins of invertebrates that have been examined seem to be collagens and that their physical properties depend upon degree of hydration. The electron micrographs of the ligament (figs. 61 and 63) do not, however, show the axial periodicity of about 640 angstrom (Å.) which is the most common characteristic of collagen fibrils (Gross, 1956). Other authors describe fibrils of 270 Å. period which participate

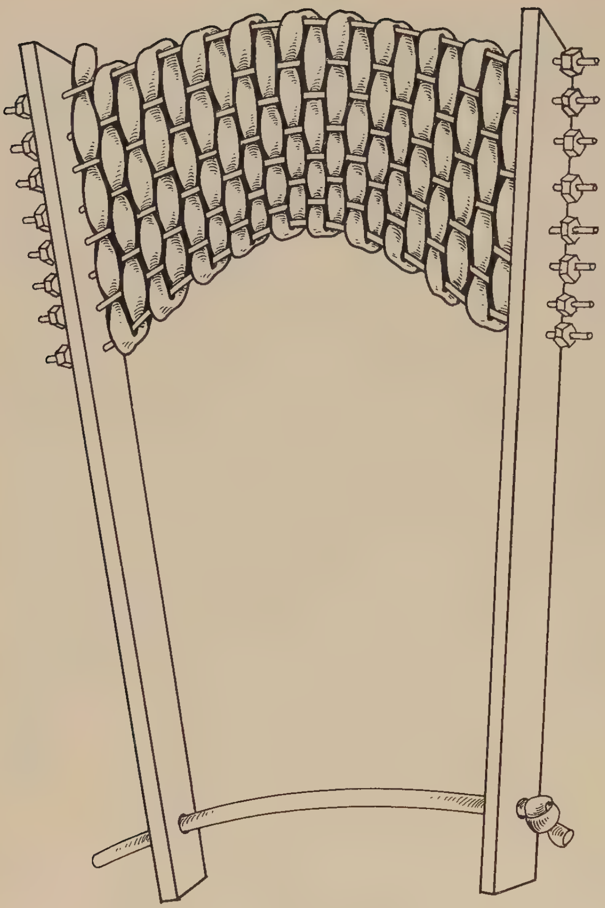


FIGURE 64.—Mechanical model of the ligament of *C. virginica.* Arches are in scale and correspond to the curves visible in a cross section of the ligament at a magnification of about 100×. Diameter of rubber tubing representing fibrillae is not in scale.

in the formation of the mature 640 Å. period collagen (See pp. 512–513 of S. L. Palay [editor] Frontiers in Cytology, 1958), as well as smaller fibrils in the embryonic tissues. The latter probably represent a very early stage in the formation of collagen.

Collagen fibers can be tanned in vitro, that is, they can be converted by various agents to a form in which they swell less and develop greater chemical resistance. The tanning of protein structures by an orthoquinone occurs naturally among many invertebrates and has been demonstrated for the cuticles of a number of arthropods (Dennell, 1947; Pryor, 1940; Pryor, Russell, and Todd, 1946) and for the chaetae of earthworms (Dennell, 1949). There is also evidence that a similar phenomenon takes place in the ligaments of bivalves (Friza, 1932). In *Anodonta*, for instance, the amber coloration of the lateral layer of the ligament is considered to be the result of tanning by an orthoquinone. This conclusion is based on the fact that even after boiling this layer induces rapid oxidation of the mixture of dimethylparaphenylenediamine and α-naphthol (Nadi reagent), which is frequently employed to indicate the presence of orthoquinones in the cuticles of insects and crustaceans (Dennell, 1947). In the ligament of *O. edulis* the differentiation between the two layers may be made visible by Mallory triple stain. The lateral layer (tensilium) consists of quinone tanned protein whereas the central layer (resilium) is built of calcified proteins (Trueman, 1951).

Few chemical studies have been made on the ligaments of oysters, but chemical analysis of the two portions of the ligament of the related pelecypod *Tellina* made by Trueman (1949) shows the following differences summarized in table 10.

It is rather surprising to find that an elastic, nonliving structure functioning through a considerable period of time (according to Trueman, several years in *Tellina*) is heavily calcified. The resilium of *C. virginica* contains a much larger amount of calcium carbonate than the outer parts: determinations made in my laboratory on the ligaments of 5- and 6-year-old oysters dried at 55° C. show that the calcium carbonate content of the resilium varied from 30 to 67 percent of the total weight of the sample, while in the tensilium the content of calcium carbonate was only from 5.3 to 8.5 percent.

It is apparent that knowledge of the chemistry of conchiolins and other substances found in molluscan shells and ligaments is incomplete and that much remains to be discovered about the composition and structure of these proteins which play such an important role in the life of all bivalves.

TABLE 10.—*Results of chemical tests of the ligament of* Tellina tenuis, *according to Trueman*

Test	Outer layer	Inner layer
Five percent HCl	No effect	
Saturated KOH (hot)	All dissolves	
Xanthoproteic reaction	+	–
Millon's reagent	+	–
Biuret reaction	–	–
Ninhydrin	+	+
Morner's reagent	+	–
Chitosan test (Campbell)	–	–
Chitin test (Schulze)	–	–
Argentaffine	Faint	–

ELASTIC PROPERTIES

It has long been known that the ligament performs a mechanical function by automatically pushing the valves apart when the tension of the adductor muscle relaxes. In a live oyster, however, the gaping of the valves never attains the potential maximum limited by the angle and length of the beaks. This can be demonstrated by a simple test: if the entire adductor muscle is severed, the valves open to a much greater angle than that maintained by a fully narcotized oyster with a completely relaxed muscle attached to the shell. It follows from this observation that during the entire life of the oyster the adductor muscle, even at the periods of its greatest relaxation, exerts a certain pulling force against the elastic tension of the ligament.

In view of the voluminous literature dealing with the structure and function of bivalve muscles it is surprising to find how little attention has been given to the study of the physical properties of the ligament. The first attempt to determine the pulling force of the muscle sufficient to counteract the elasticity of the ligament was made in a rather crude manner in 1865 by Vaillant who tried to measure the elastic force of the ligament of *Tridacna* shells. Trueman (1949) erroneously gives credit for this pioneer work to Marceau (1909), who only repeated the method used by earlier investigators (Plateau, 1884).

After removing the soft body of *Tridacna*, Vaillant set the empty shell on a table with the flat valve uppermost and placed a glass graduate on top of it. Water was poured into the graduate until the valves closed. Then the volume of water was read and its weight computed. The weight of the water plus the weight of the glass container and of the valve gave Vaillant a value which he called the resistance of the ligament. For a shell of *Tridacna*, apparently one of small size, he gives the following figures: weight of water required to close the valves—250 g.; weight of the vessel—700 g.; weight of the valve—632 g. The total force needed to overcome "the resistance" of the ligament is, therefore, 1,582 g.

A similar method was used by Plateau (1884), the only differences being that weights were added to a metal pan suspended from a loop encircling the valves, as shown in figure 65, and that the shell was placed on a metal ring. The elastic force exerted by the ligaments of several common bivalves, as determined by Plateau, was found to be as follows: *Ostrea edulis*—333.8 g.; *Venus verrucosa*—500.0 g.; *Mya arenaria*—620.0 g.; and *Mytilus edulis*—1,051.8 g. In Marceau's paper of 1909 the data taken from Plateau's work are repeated without change or verification.

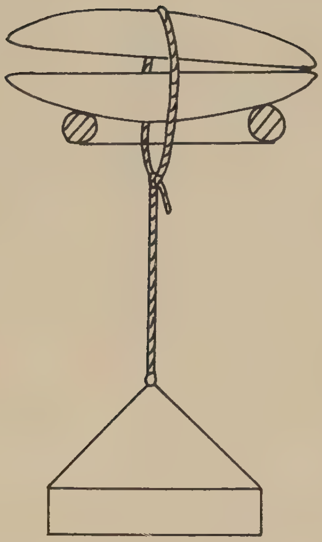


FIGURE 65.—Plateau's method of measuring elasticity of the ligament.

Trueman's investigation of the ligament of *Tellina* (1942) marks a renewal of interest in the study of the physical properties of the ligament. In a later paper (1951) he finds that in very young *O. edulis* the outer layer of the ligament (tensilium according to our terminology) forms a continuous band along the entire dorsal margin of the hinge, but that in adults this outer layer separates into the anterior and posterior portions, leaving the inner layer (resilium) exposed at the dorsal edge. The axis about which the valves of the adult *O. edulis* open (pivotal axis) is the same in *C. virginica* (figure 54, piv. ax.). In the closed shell of *Ostrea* and *Crassostrea* the central part of the ligament (the resilium) is under compression and the two flanking portions (tensilium or outer layer of Trueman) are under tension.

To measure the opening moment of thrust of a hinge ligament, Trueman (1951), uses the following method, shown diagrammatically in figure 66: soft parts of the body are removed and the lower valve embedded in plasticine; a counterbalanced beam is erected above the valve in such a way that the weight placed on the pan at the left end is applied at the center of the upper valve. The distance from the left end of the beam to the arm touching the centroid of the

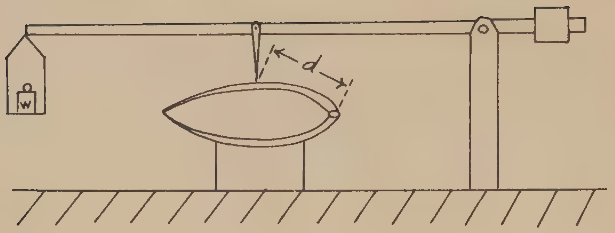


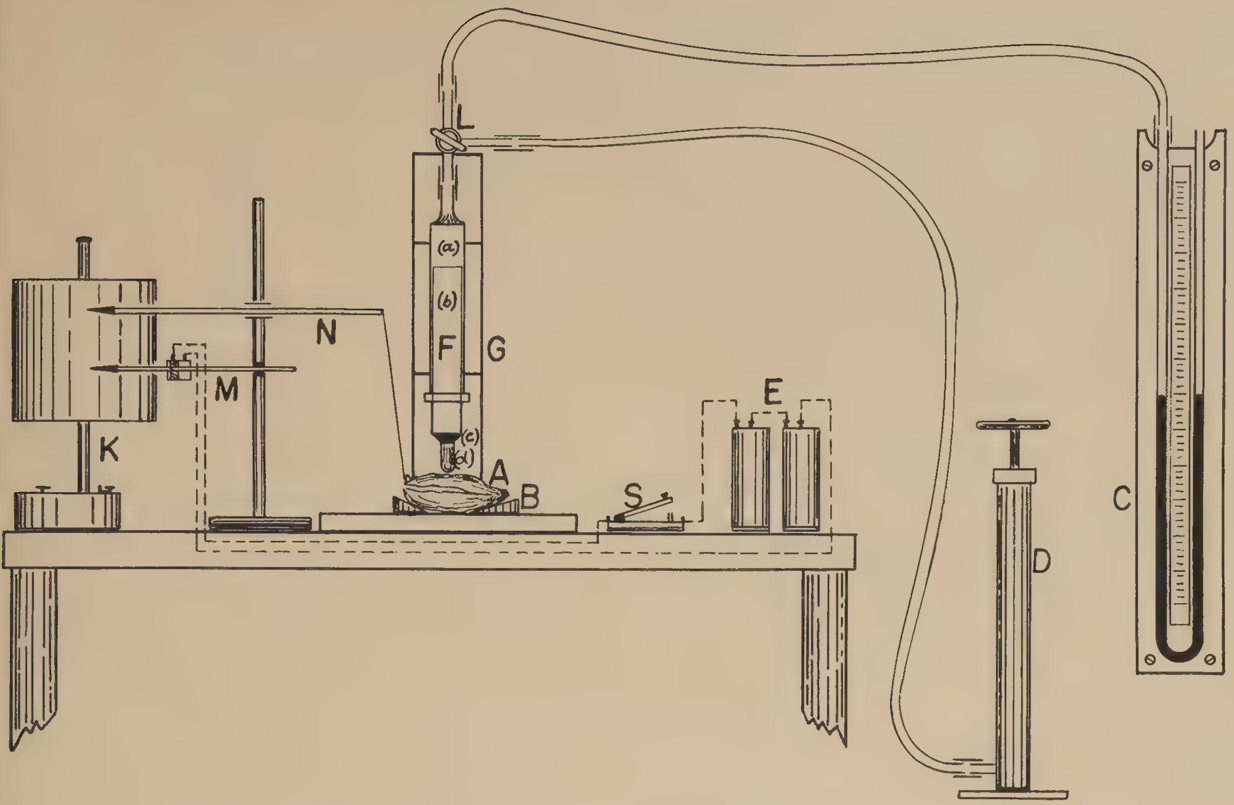
Figure 66.—Trueman's method for measuring the moment of thrust of bivalve ligament. Redrawn from Quarterly Journal of Microscopical Science, 1951, series 3, vol. 92, part 2, p. 137.

valve is so adjusted that the weight at the point of application to the centroid is twice that placed on the pan. Weights are gradually added until the valves just close so that the opening moment M is exactly counterbalanced. The ratio M′ between the opening moment M and the surface area of the valve A is determined by the formula: $M' = d\frac{(2W+V)}{A}$, where d is the straight line distance from the point of weight application on the shell to its pivotal axis; W is the weight applied; and V is the weight of the upper valve.

There are two objections to this method. The central point of the valve can be accurately determined only for round, symmetrical shells; for the irregularly curved shells of *C. virginica*, *C. angulata*, or *C. gigas*, its position can only be guessed. Another more serious objection refers to the determination of the weight under which the valves "just closed." Experimenting with *C. virginica*, I found that visual observation, even with a magnifying glass, is not sufficient to determine when the valves are completely closed. Frequently a tiny slit between the valves cannot be seen but becomes apparent on a magnified kymograph record of shell movement. Trueman's method with modifications was used by Hunter and Grant (1962) to study the mechanical characteristics of the ligament of the surf clam, *Spisula solidissima*. They found that the ligament of the clam is about 3.5 times stronger (in terms of opening moments) than that of *Mya arenaria*. The mechanical differences, according to their opinion, reflect the modes of life of the two clams.

The moment of thrust measured by Trueman's method is of no particular significance to the physiology of the oyster because it does not represent the pulling force which the adductor muscle must exert to close the valves or to keep them partially open. This force differs from Trueman's moment of thrust because the site of the attachment of the adductor muscle is located not in the center but in the ventroposterior quadrant of the valves. The following method overcomes these difficulties: the body of the oyster is removed without injuring the ligament; the gaping shell is placed with the left valve resting on concave cement support (fig. 67) and immobilized by small lead wedges. The right valve is connected to writing lever *N* of kymograph *K*. A glass hypodermic syringe of 10 ml. capacity, mounted on wooden frame *G*, is placed so that its plunger *F* touches the valve over the center of the muscle attachment area. The flattened end of the plunger is cut off, and its stem is sharpened to a point. A three-way stopcock *L* is attached by hard rubber tubing to the upper end of the syringe; one of its arms is connected to a hand pump *D* (automobile or bicycle tire type); the other arm leads to an open mercury manometer *C*. Two dry cell batteries *E* activate the recording electro-magnet *M* which makes a mark on the drum only when the key switch *S* is pushed down. As the pump is worked the pressure created in the system forces the plunger down, gradually closing the shell. Each time the mercury column rises 2 mm. the operator pushes the signal key down. Pumping is continued after the valves are closed until the horizontal line on the drum record indicates that increase in pressure produces no further change in the position of the upper valve. The point corresponding to the complete closure of the valves is easily determined by placing a ruler against the horizontal portion of the kymograph curve and noting the point at which the line begins to curve down (fig. 68). The number of signal marks from the beginning of the recording to the end of the curved line multiplied by two gives the height of the mercury column in millimeters. The manometer must be calibrated to correct for the error resulting from slight irregularities in the diameter of the glass tubing in its two arms.

To minimize friction between the walls of the syringe and its piston, several lubricants were tried until it was finally discovered that a minute quantity of high-speed centrifuge oil permits free movement of the piston under its own weight. The weight of the piston in the operating position, determined by placing the balance pan under the point of the piston, was recorded at 17.0g.; weight of the same piston taken out of the syringe was



IGURE 67.—Apparatus used for determining the elastic force of the ligament. A—oyster shell; B—support; C—mercury manometer mounted on wall; D—air pump; E—two dry cells to operate the signal magnet M; F—plunger of hypodermic syringe resting on right valve above muscle attachment area; G—stand upon which the syringe is mounted; K—kymograph; L—three-way stopcock; M—signal magnet with writing pen; N—lever connected to upper valve of the oyster A; S—key switch for signal magnet.

8.45g. Both syringe and piston were cleaned and ubricated at the beginning of each series of ob-ervations and the weight of the piston in the perating position checked frequently. Prior nd during the determination, which required nly a few minutes, the ligament was kept moist y frequent applications of a few drops of sea ater.

To convert the manometer readings into force grams, the following simple computation was ade: since the cross-section area of the piston the syringe is 1.971 cm.^{2} and the specific gravity f mercury is 13.95, the weight of the column of ercury is equal to 1.971 x 13.95 x H where H the height of that column in centimeters. eterminations of elastic force made by this ethod are accurate within 5.3g. since readings ere taken at 2 mm. intervals and the weight f a mercury column of 1 cm. height is 26.71g.

With exposure to air the elasticity of the liga-ent changes, gradually losing its resilience. As drying progresses greater force must be applied to bring the valves together, and the ligament becomes harder and more brittle until it finally breaks along the pivotal axis. The rate of these changes was ascertained in two tests with large American oysters from Peconic Bay, N.Y. After the shell was placed in the apparatus (fig. 67) determinations were made at 15-minute intervals between which the ligament was not moistened. Room temperature varied slightly from 68° to 70° F., and relative humidity in the laboratory was 46 percent. The results of testing which continued for 5 hours and 5 minutes indicate that under the conditions of the experiment no significant change in the physical properties of the ligament is noticeable during the first 90 minutes. After that the hardness of the ligament increases steadily as can be seen from the shape of the curve in figure 69. The test repeated a second time yielded similar results. It can, therefore, be deduced that under the given experimental condi-

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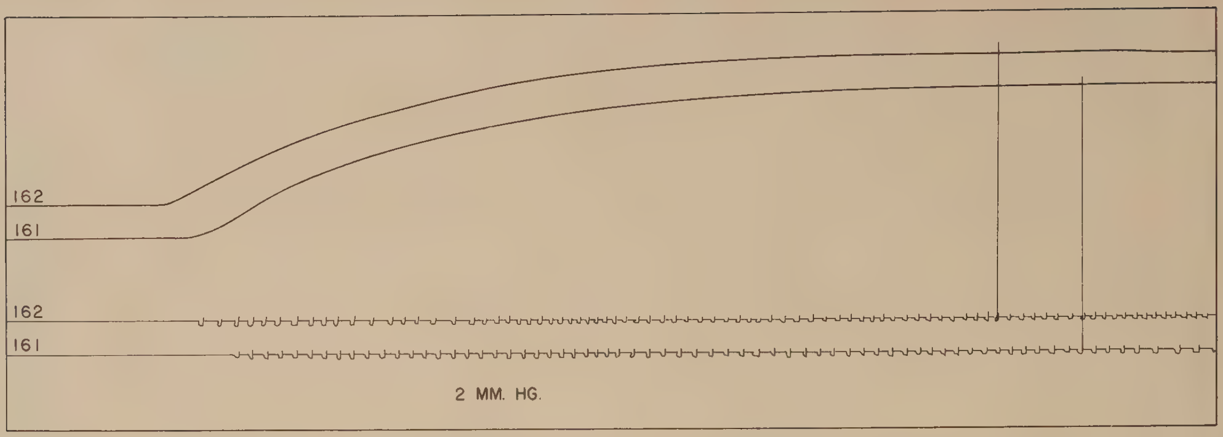


FIGURE 68.—Two kymograph records of the closing of oyster valves under pressure applied at the upper valve over the muscle attachment area. Marks on the bottom lines refer to each 2 mm. increase in the height of the mercury column in the manometer. Vertical lines indicate the point on the abscissa at which the final reading was made.

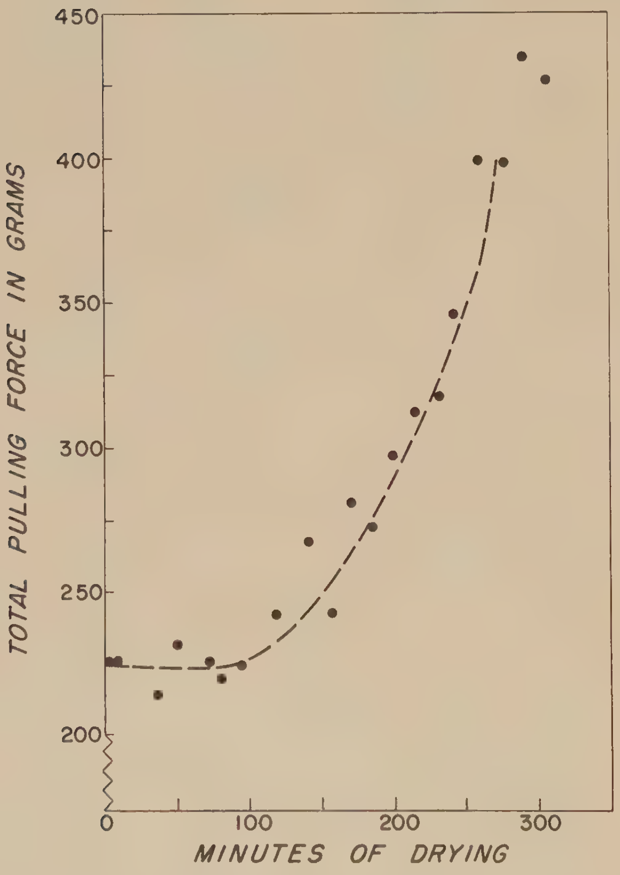
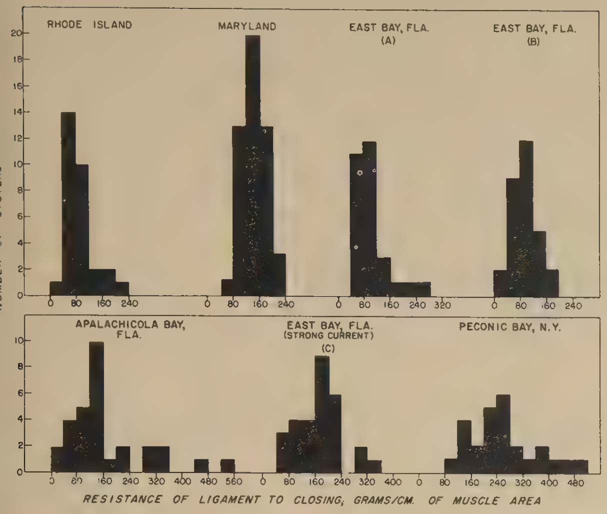


FIGURE 69.—Effect of drying on elasticity of the ligament of adult *C. virginica* from Peconic Bay, New York. (At temperature of 68° F.)

tions drying can not affect the values of readings obtained within a few minutes after the removal of the shells from water.

The question arises whether there are significant differences in the elastic properties of the ligaments of oysters living in different ecological environments. The problem was studied by obtaining samples of oysters from the following localities: Peconic Bay, N.Y. (nearly oceanic water of high and stable salinity); upper part of Narragansett Bay, R.I. (18°/oo to 24°/oo); Chesapeake Bay, Md. (10°/oo to 16°/oo), both localities characterized by considerable daily and seasonal fluctuations in salinity of water; Apalachicola Bay and East Bay, Fla., representing typical southern conditions of warm water and great fluctuations in salinity. Oysters from East Bay (near Pensacola, Fla.) were taken from three different zones: A—intertidal flat; B—bottom level; and C—below low water level in the area of exceptionally strong tidal currents. Each sample consisted of either 30 or 50 adult oysters of marketable size. After arrival at Woods Hole, Mass., they were kept at least 5 weeks in the harbor water (31°/oo to 32°/oo) before they were tested. All experiments were conducted during the winter when harbor water temperature was about 4° C. and laboratory air temperature about 21° C.

The results of the tests, expressed as the pulling force in g. per cm.^{2} of the muscle scar area necessary to counteract the elasticity of the ligament, are summarized in the series of histograms shown in figure 70. It is apparent that the elastic properties of the ligament vary greatly within each group but especially in the Peconic Bay and Apalachicola oysters. A comparison of the modes of the elastic forces in ligaments of oysters from different environments gives the following values



IGURE 70.—Frequency distribution of the elastic property of the ligaments in seven groups of adult *C. virginica.* The elastic property is expressed in the pulling force of the adductor muscle (in g. per cm.^{2} of muscle area) needed to counteract the action of the ligament.

xpressed in g. per cm.^{2} of transverse section of uscle area arranged in diminishing order:

Peconic Bay (Fireplace oysters)	252g.
East Bay, Fla.—C, fast tide	178g.
Apalachicola Bay	128g.
Chesapeake Bay, Md	99g.
East Bay, Fla.—B, bottom	93g.
East Bay, Fla.—A, intertidal zone	91g.
Narragansett Bay	79.g

Vhether the values observed do actually depend n ecological conditions cannot be stated without rther investigation.

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CHAPTER IV

GENERAL ANATOMY

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Before proceeding with the detailed description of the structure and function of various organs in the oyster, it appears desirable to present a general anatomical picture of this mollusk and to show the arrangement and topography of the various systems of organs.

The anatomy of edible oysters is described in several papers. Brooks (1905), Moore (1898), Churchill (1920), and Galtsoff (1958) give general accounts of the anatomy of *C. virginica*. The structure of the European oyster, *O. edulis*, is described by Orton (1937), Ranson (1943), and Yonge (1960); a brief and partial description of the anatomy of *C. angulata* by Leenhardt (1926) ncludes the histology of the species. The structure of the Bombay oyster, *O. cucullata*, is described by Awati and Rai (1931); and a short anatomical sketch of the Australian oyster, *O. commercialis*, s given by Roughley (1925).

The anatomical sketch of an adult *C. virginica* given in this chapter describes the principal organs of the oyster as they can be seen by disecting the mollusk and examining the preparation nder a low-power microscope. Details that an be observed by sectioning, staining, and econstruction are described in the chapters of his book dealing with the respective organ ystems.

METHODS OF STUDY

Successful dissection of the oyster depends to a onsiderable degree on the condition and shape of he specimen selected for study. It is convenient o work with broad and large oysters measuring bout 4 to 5 inches in height and containing lean meats. In fat or in sexually mature specimens the large quantities of glycogen and of sex cells covering the organs make their anatomy difficult to trace. Oysters most suitable for anatomical study are those which have completed spawning but have not yet accumulated much glycogen. In New England waters such oysters can be found in September and early October.

For dissection the oyster should be opened by removing its flat (right) valve, a process facilitated by first narcotizing the oyster. Narcosis eliminates the necessity of forcibly prying apart the valves, a manipulation which in the hands of an inexperienced person frequently results in injury of the underlying tissues or, even more often, to the hand of the operator by the sharp edge of the shell. Another advantage of working with a fully narcotized specimen is that the organs and tissues remain fully expanded in their normal position and are undistorted by contraction.

The best method of narcotizing is to use technical magnesium sulfate (Epsom salt) as follows: The oyster is thoroughly washed and scrubbed to remove fouling organisms and then placed in a suitable container, about 8 to 9 inches in diameter and 3 inches deep, filled with sea water. During the first 24 hours small quantities of Epsom salt are gradually added until a concentration between 5 to 10 percent is reached, then the oyster is left undisturbed for another 24 or even 48 hours at room temperature. The magnesium sulfate should be added very gradually because an excess of it at the beginning of narcosis may cause the oyster to close its valves and thus prolong the process. Additional amounts of salt may be added because oysters tolerate much higher concentrations of magnesium sulfate and recover from it upon being placed in running sea water. Completely narcotized oysters do not respond to touch or prick at the edge of the mantle.

With the narcotized oyster grasped in the hand, right valve uppermost, a knife is inserted between the mantle and the shell and carefully pushed above the meat toward the adductor muscle, its edge always at a sharp angle to the inner surface of the valve. Actually it is preferable to move the oyster right and left, while gently pressing it against the edge of the knife, rather than to move the knife itself. After the attachment of the adductor muscle to the shell is severed, the flat, right valve is lifted up until the ligament breaks and the oyster is exposed in the cupped valve which retains sea water. (In oyster bars and restaurants raw oysters are usually served on the right (flat) valve and the cupped left valve is removed.) If it is necessary to open an unnarcotized oyster, I prefer first to break the ligament with a screwdriver, then to lift the valve carefully and cut the adductor muscle. This method reduces the chances of cutting the visceral mass.

Dissection is much facilitated by allowing the tissues to harden in 3 percent formalin for at least 1 day. For tracing the digestive tract and the blood vessels I recommend, furthermore, the injection of these systems with colored moulage latex. For study of the digestive tract the following method gives satisfactory results: the mouth of the oyster is exposed by cutting out a small triangular section of both valves and pushing up the underlying tissues (the mantle cap). Blue or red latex diluted with about 20 percent water is injected into the mouth through a wide glass pipette slightly flattened at the tip and supplied with rubber bulb. During the injection the oyster is held in a vertical position. Sometimes it is difficult to fill the entire digestive tract with latex injected through the mouth. An additional injection can then be made through the anus with a 2-ml. capacity hypodermic syringe, preferably one of metal since latex rapidly adheres to glass and causes the plunger to stick.

For injecting blood vessels through the ventricle or auricles I prefer to use either latex diluted with about 30 percent of water or vinyl resin solution. Injection should be completed without interruption in one operation, after which the injected specimen is immediately placed in 5 percent formalin in tapwater and left undisturbed for several hours or overnight to allow complete setting of the latex or plastic. Preparations may be indefinitely preserved in 3 to 5 percent formalin.

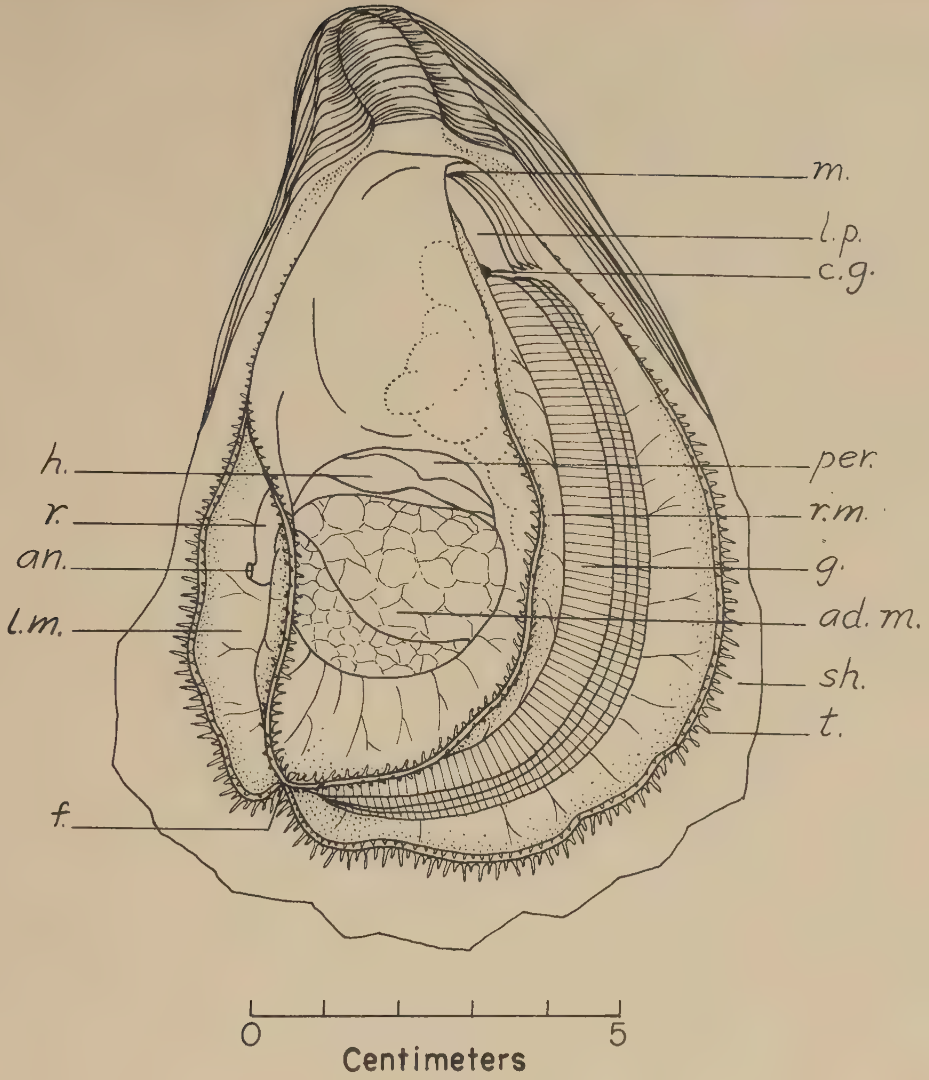
Various cavities and chambers of the oyster body can be advantageously studied by making plaster of paris casts. The valves of a live oyster are forced apart by inserting an oyster knife at the ventroposterior margin of the shell and gradually rotating the knife until its edge is perpendicular to the surface of the valves. The valves should be opened very slowly to avoid tearing the adductor muscle. After a small wooden wedge is inserted to prevent closing of the valves, freshly made plaster of paris paste of the consistency of heavy cream is injected into the cloaca and into the opening of the promyal chamber. From time to time the injected specimen is tapped gently against the table to insure complete penetration of the plaster into the smallest ramification of the gill tubes. After the filling with plaster of paris is completed, the wedge is removed and the valves are pressed together. The preparation is left undisturbed for 24 hours. After the plaster of paris has hardened the shells and the soft parts of the body are removed, the cast is dried for 24 hours at 56° C. and finally may be dipped in a hot mixture of beeswax and turpentine to prevent breaking of the finest ramification of the replicas.

ORGANS UNDERLYING THE SHELL

After the valve is removed, the body of the oyster is seen to be covered with a soft membrane called the mantle (figs. 71, 72). The mantle is a bisymmetrical organ. Its left and right folds are joined together at the dorsal edge where small and slightly pigmented fold (not shown in the figure) marks the position of the ligamental ridge, a special organ which secretes the ligament. The joint portion of the two lobes forms a cap which covers the mouth and its associated structures (fig. 71, m.). The remaining mantle edges are free except for a point at the extreme ventral margin (f.) where the two opposing lobes are fused together to form a wide funnel-like channel, the cloaca (fig. 72, cl.).

The edge of the mantle consists of three protruding fringes, two of which, the outer and the middle, are beset with highly sensitive tentacles (t.). The tentacles and the edge of the mantle are commonly pigmented.

The parts of the mantle not attached to underlying organs enclose a large space filled with sea water and known as the mantle cavity. Sometimes the open space under the mantle is referred to as the shell cavity and the sea water retained in it as the shell liquor. The space between the mantle and the gills is often called the "gill cavity,"



IGURE 71.—Organs of *C. virginica* seen after the removal of right valve. ad.m.—adductor muscle; an.—anus; c.g.—cerebral ganglion; f.—fusion of two mantle lobes and gills; g.—gills; h.—heart; l.m.—left mantle; l.p.—labial palps; m.—mouth; per.—pericardium; r.—rectum; r.m.—right mantle, sh.—shell; t.—tentacles. The right mantle contracted and curled up after the removal of the right valve, exposing the gills. Portion of the mantle over the heart region and the pericardial wall were removed. Drawn from live specimen.

n undesirable term because of possible confusion rith the inner spaces (cavities) of the gills. The orrect terminology for the latter is water tubes nd gill chambers. The expression mantle cavity eems to be more appropriate than the shell avity. As to the shell liquor, the term is well stablished, especially in papers dealing with the acteriology of the oyster and, therefore, it should e retained.

Under normal conditions the mantle underlies nd adheres slightly to the shell, the secretion of hich is its principal function. As will be shown ter, this organ also participates in several other functions: it controls the flow of water for respiration and feeding; plays an important role in female spawning; and receives and transmits sensory stimuli.

In a living oyster the mantle cavity is always full of sea water. As the shell is closed the surplus water is ejected, but that remaining in the free spaces between the mantle lobes (fig. 73, m.c.) keeps the enclosed organs constantly bathed in water.

Various products of oyster metabolism accumulate in the shell liquor as well as considerable quantities of mucus and blood cells discharged

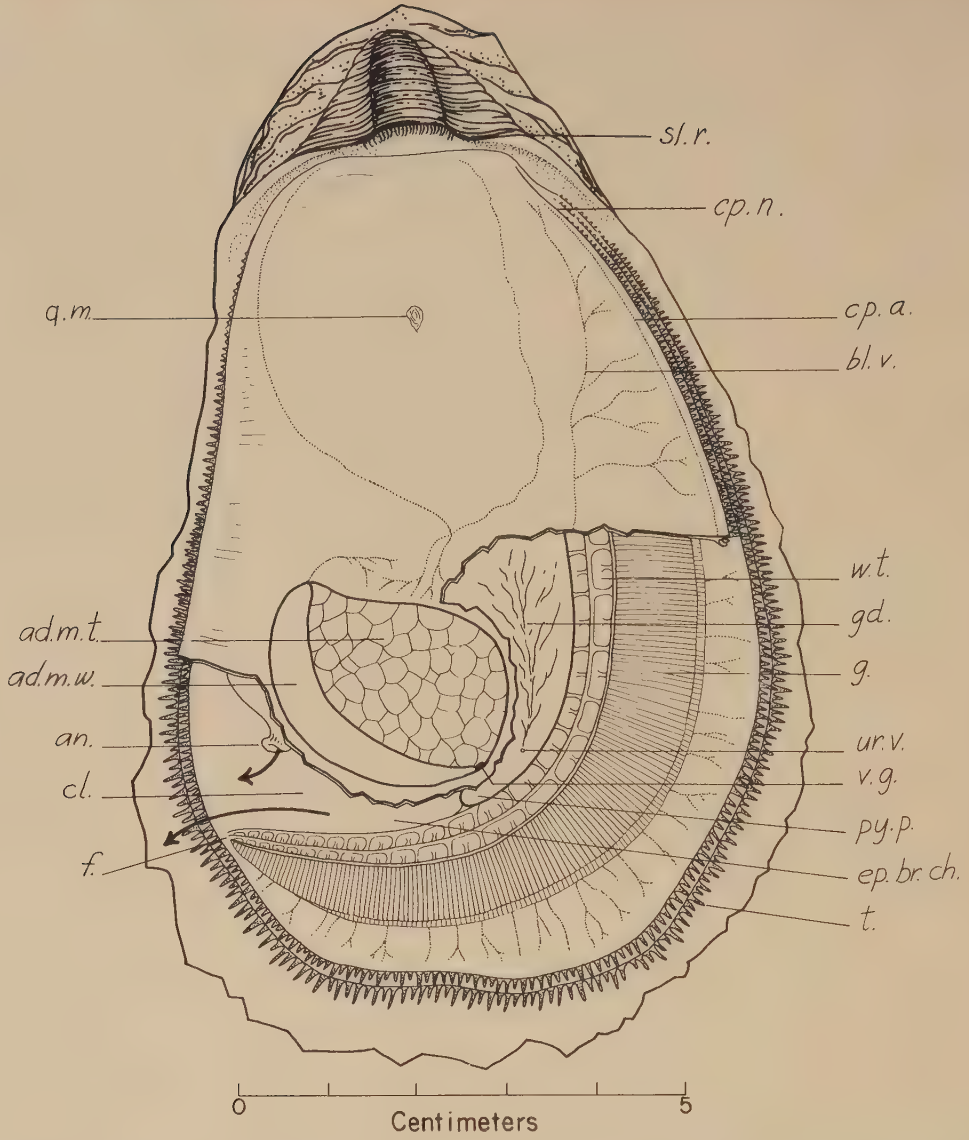
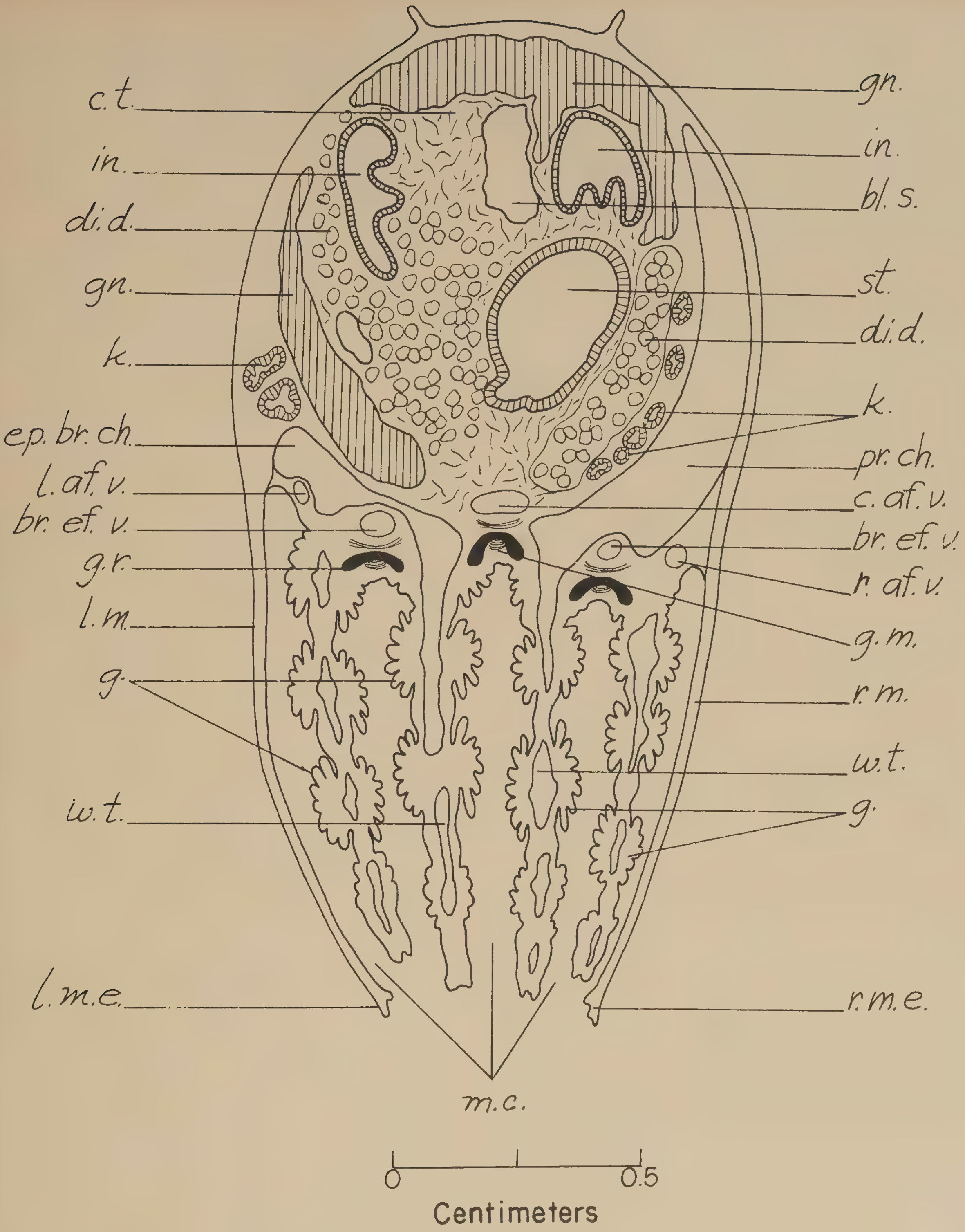


FIGURE 72.—Oyster viewed from the right side. Portion of the right mantle and the wall of the epibranchial chamber cut off to expose the gills and their water tubes, the cloaca, and the lower part of the gonad. ad.m.t.—adductor muscle, translucent part; ad.m.w.—adductor muscle, white (opaque) part; an.—anus; bl.v.—blood vessels of the mantle; cl.—cloaca (the arrow indicates the direction of the outgoing current of water); cp.a.—circumpallial artery; cp.n.—circumpallial nerve; ep.br.ch.—epibranchial chamber of the gills; f.—fusion of gills and mantle; g.—gills; gd.—gonoducts; py.p.—pyloric process; q.m.—rudimentary Quenstedt's muscle; t.—tentacles; ur.v.—opening of the urinogenital vestibule; v.g.—visceral ganglion; w.t.—water tubes of the gills. Drawn from a preserved specimen.

through the mantle and gills. The alkalinity of the shell liquor retained in the mantle cavity therefore decreases with time as the oyster remains closed. Although liquor may become slightly acid, excessive acidity is stopped by the buffering action of calcium carbonate dissolved from the shell.

The ability of the oyster to retain shell liquor is a useful adaptation to life in the intertidal zone permitting the animal to survive many days of exposure to air. It is equally useful to those oysters which live below the low water mark and are never exposed to air. By closing their shells tightly and by retaining some sea water they are able to survive unfavorable conditions caused by floods or by the temporary presence of toxic or irritating substances in the water.

The color of the surface of the mantle facing the shell is variable. Lean oysters devoid of glycogen are usually of a dull grayish color whereas "fat"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IGURE 73.—Transverse section of the dorsal part of an adult *C. virginica* a short distance below the labial palps. Drawn semidiagrammatically from an enlarged photograph of stained section. Bl.s.—blood sinus; br.ef.v.—branchial efferent vein; c.af.v.—common afferent vein; c.t.—connective tissue; di.d.—digestive diverticula; ep.br.ch.—epibranchial chamber; g.—gills; g.m.—gill muscles; g.r.—gill rod, gn.—gonad; in.—intestine; k.—kidney; l.af.v.—lateral afferent vein; l.m.—left mantle; l.m.e.—edge of left mantle; m.c.—mantle cavity; pr.ch.—promyal chamber; r.af.v.—right afferent vein; r.m.—right mantle; r.m.e.—edge of right mantle; st.—stomach; w.t.—water tube of the gills.

oysters are white and those full of spawn are creamy-yellowish. The green color of oysters from certain localities is attributed to the accumulation of copper or to the absorption of blue-green pigment from certain diatoms upon which they feed. Mantle color is always a good indication of the condition of the mollusk.

Several ramifying blood vessels can be easily seen on the surface of the mantle (fig. 72, bl. v.). A broad blood vessel along the periphery is the circumpallial artery (fig. 72, cp. a.). A narrow and darker line immediately adjacent is a circumpallial nerve (fig. 72, cp.n.). A small, slightly pigmented depression in the dorsal end of the mantle marks the position of a nonfunctional Quenstedt's muscle (fig. 72) barely attached to the valve.

The most conspicuous element of the oyster anatomy visible after the removal of the valve is the posterior adductor muscle. This ovate organ consists of a larger dorsal, translucent portion (fig. 72, ad.m.t.) and a consistently smaller, ventral opaque part (ad.m.w.).

A semitransparent oval membrane covers the pericardium, the chamber in which the heart is suspended (fig. 71, per.). On the left side of the oyster the pericardial wall lies directly under the valve, but on the right side the large and asymmetrical promyal chamber (fig. 73, pr.ch.) separates the pericardium from the mantle.

ORGANS UNDERLYING THE MANTLE

Directly under the free edge of the mantle along the entire anteroventral side of the oyster lie the gills (fig. 72, g.). They can be exposed by cutting off the mantle along the line of its attachment to the base of the gills, or by lifting the mantle and pulling it up. If a piece of shell is sawed off at the anterior edge of the valve the corresponding portion of the mantle curls up and exposes the gills underneath. For several days the opposite fold of the mantle retains its normal position with the tentacles (t.) spread over the edge of the shell, while the curled mantle edge under the cut secretes a vertical plate. Later on the mantle fold of the intact side of the oyster also curls up and by depositing new shell material at the angle to the valve closes the gap. Cutting off a portion of one valve proved to be a useful procedure for observing the functions of the gills and mantle.

The gills consist of two pairs of lamallae or gill plates, one pair on each side (figs. 71, 72, g.). At the anterodorsal margin their free and gently curved edges touch the lower tips of the labial palps (fig. 71, l.p.) and their bases are joined to the mantle. In the ventroposterior part of the body the gills and the two lobes of the mantle join to form a channel (fig. 71, f.) which marks the entrance to the cloaca (fig. 72, cl.).

The mouth (fig. 71, m.), a narrow horizontal slot above the dorsal edges of the two posterior labial palps, lies under the hood or cap formed by the anterior fusion of the two mantle folds. It can be seen by cutting off the mantle cap and pressing down the upper (dorsal) edges of the palps.

The cloaca (fig. 72, cl.), a large funnel-shaped space between the ventral side of the adductor muscle and the gills, is a continuation of the epibranchial chamber (fig. 72, ep.br.ch.) which extends along the gills. The latter can be exposed by cutting along the wall of the cloaca, starting from the mantle junction (fig. 71, f.) and following the edge of the muscle. The epibranchial chamber extends along the base of the gills. When the dissected portions of the cloacal wall are pulled apart, the following structures are revealed: the rectum and anus (figs. 71, 72, r., an.), located on the ventroposterior border of the adductor muscle; the blunt tip of the pyloric process (fig. 72, py.p.) of the visceral mass, which projects into the epibranchial chamber; the small and almost invisible opening of the urinogenital groove or vestibule (fig. 72, ur.v.), located on the wall of the pyloric process; and the visceral ganglion (fig. 72, g.), situated in a shallow depression between the two divisions of the adductor muscle and partially covered by the pyloric process.

The heart (fig. 71, h.), seen after removal of the pericardial wall, consists of one ventricle and two pigmented auricles. Two aortae (not shown in the diagram) emerge from the tip of the ventricle, and large venous sinuses (also not shown) empty into the auricles. The slightly pigmented structure extending dorsally from the auricular side of the pericardium along the base of the gills is the organ of excretion (kidney) frequently called the organ of Bojanus (fig. 73, k.). Inasmuch as there is no doubt regarding the function of this tubular thin-walled organ it seems preferable to call it the kidney. Urine is collected in a large reservoir in the lower (ventral) part of the kidney before being discharged through the urinogenital vestibule. On each side of the auricular part of the pericardium there is a fine opening from which a canal leads to the kidney. This renopericardial opening and the canal are difficult to see with the unaided eye.

THE VISCERAL MASS

The very short esophagus enters the large and somewhat twisted stomach, into which a wide sac containing the crystalline style also opens. The visceral mass (figs. 72, 73) occupies the dorsal half of the body, above the adductor muscle. It consists of esophagus, stomach, crystalline style sac inside the pyloric process, and intestine embedded in connective tissue. The stomach directly communicates with the digestive diverticula, a greenish mass of glandular tissue (fig. 73, st., di. d.), which completely surrounds both stomach and intestines. The intestine, after leaving the stomach, makes a loop (fig. 73, in., and fig. 197, ch. X) which ends in the rectum (fig. 71, r.) at the dorsal edge of the adductor muscle. A small rosette at the tip of the slightly protruding rectum surrounds the anus (fig. 72, an.), located in the area continually swept by the current of water from the cloaca (fig. 72, cl.).

Between the digestive diverticula and the surface epithelium lie the gonads (fig. 73, gn.). After spawning these layers disappear almost completely, being represented only by a thin germinal lining. The gonad is not visible to the unaided eye at this stage. At the time of full sexual development the layer of gonadal tissue in large specimens may reach several millimeters in thickness. Many branching channels, the gonoducts (fig. 72, gd.), through which sex cells are discharged, are clearly visible on the surface of a sexually mature specimen. They all empty into a common gonoduct leading into the urinogenital groove (fig. 72, ur. v.) from which eggs or sperm are discharged into the epibranchial chamber (ep. br. ch.). Secondary sex characters are absent.

The sex of the oyster can be recognized by microsocopic examination of the gonad. Hermaphrodites among adult *C. virginica* are rare. Out of many thousands of oysters examined in the course of my studies I have found only one oyster with the gonads containing both eggs and sperm. The European oyster, *O. edulis,* and the Olympia oyster, *O. lurida,* are hermaphroditic. (A full discussion of sex in the oyster is found in chapter XIV.)

The position of the fully developed gonad in relation to other organs of the visceral mass can best be studied in a series of transverse sections of the dorsal half of the oyster. Figure 73 shows the relative position of the organs as seen on the section made just below the labial palps. The gonad (gn.) is irregular in shape and located close to the surface of the body. The digestive diverticula (di. d.) occupy the larger part of the visceral mass between the gonad and the digestive tract itself, which at this level is represented by the stomach (st.) and two cross sections of the intestines (in.). The rest of the visceral mass consists of connective tissue (c.t.) containing irregular blood sinuses (bl. s.), which may be full of blood. The series of twisted tubules comprising the kidney (k.) is located near the surface on both sides of the body above the gills.

The large empty chambers between the gills and the visceral mass directly communicate with the water tubes (w.t.) of the gills (g.). The epibranchial chamber (ep. br. ch.) on the left side is much smaller than the corresponding chamber on the right side. The latter, called by Nelson (1938) promyal chamber (pr. ch.), extends to the dorsal end of the oyster and opens to the outside in the posterodorsal part of the body, independently of the cloaca. Water tubes (w.t.) inside the gill plates open into these chambers. Soft and delicate tissue of the gills is supported by the framework of chitinous rods, the largest being located at the base of the gills (g.). Two sets of muscles (g.m.), one below and one above the largest gill rods, control the movements of the plates. The five principal blood vessels of the gills are located above the skeletal rods: the common afferent vein (c. af. v.); two branchial efferent veins (br. ef. v.), one on each side; and two lateral afferent viens (l. af. v.), also one on each side of the oyster.

NERVOUS SYSTEM

The nervous system can be studied best by reconstructions made from sectioned material since only the principal nerves and ganglia can be revealed by dissection. The visceral ganglion (fig. 72, v.g.) is located in a slight depression on the anterior side of the adductor muscle, partially hidden by the tip of the pyloric process. It can be observed by cutting the wall of the cloaca and of the epibranchial chamber, then placing the oyster on its posterior edge, lifting its ventral side slightly toward the observer, and pulling the dissected portions of the wall apart. The ganglion and its nerves are then visible against the background of the surrounding tissues. Some of the individual nerves, namely, the posterior pallial nerve (fig. 253, in ch. XIII, p.p.n.) and the lateral pallial nerves (l.p.n.), emerge from the posterior end of the ganglion and can be followed without much difficulty until they begin to ramify. The posterior pallial nerve follows the right side of the adductor and sends a short branch to a sense organ—a small unpigmented protuberance called the pallial or abdominal organ (p.o.). On the left side of the oyster the pallial organ is much smaller and is located much closer to the ganglion; in fact, in many oysters only the right pallial organ is present.

The anterior pallial nerve (fig. 253, in ch. XIII, a.p.n.) and branchial nerve (br.n.) leave the dorsal end of the ganglion and for some distance follow the nerve trunk which leads to the cerebral ganglia and is known as the cerebrovisceral connective (c.v.con.). The cerebral ganglia (fig. 71, c.g.) are embedded in connective tissue at the bases of the labial palps. A very thin cerebral commissure passes dorsally over the esophagus and connects the cerebral ganglia in a typical loop or ring. The circumpallial nerve (fig. 72, cp.n.) follows the circumpallial artery and can easily be seen at the edge of the expanded mantle. The other nerves emerging from the visceral and cerebral ganglia can be more conveniently studied on sectioned preparations and are described in chapter XIII.

ANATOMICAL PECULIARITIES

In several respects the anatomy of the oyster is simpler than that of other bivalves. The absence of a foot results in the lack of pedal ganglia; only the posterior adductor muscle is present, and there are no specialized organs of sight, although the animal is sensitive to change of illumination. On the other hand, the edge of the mantle is fringed with highly sensitive tentacles abundantly supplied with nerves leading to the ganglia. As in other bivalves, the nervous system is not centralized but is represented by widely separated ganglia. The structure of the cerebrovisceral connectives, of the circumpallial nerve, and other large nerves resembles more the structure of ganglia than that of the nerve, a condition which undoubtedly results in a high degree of coordination among the various parts of the organism.

In addition to performing their principal functions, several organs of the oyster also participate in other activities. The gills, for instance, are not only the organ of respiration but collect and sort food as well. The mantle is used extensively in the control of the flow of water through the body; the coordinated action of the adductor muscle, gills, and gonad is necessary for the effective discharge of eggs by the female oyster. In other animals such functions are performed by special organs, but in the evolution of the oysters the high degree of coordination developed among different parts of the body eliminates the need for specialized structures, and new and complex functions are successfully performed by synchronizing the work of the existing parts.

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CHAPTER V

THE MANTLE

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The inner organs of all mollusks are covered with a soft and fleshy fold of tissue called the mantle or *pallium* (Latin for cloak or coverlet). The structure of the mantle is relatively simple: the organ consists of a sheet of connective tissue containing muscles, blood vessels, and nerves and is covered on both sides by unicellular epithelium. Many blood cells invade and wander throughout the entire thickness of the mantle, infiltrating the spaces (sinuses) in the connective tissue, and crawling through the epithelium to aggregate on the outer surface of the mantle.

Although the principal role of the mantle is the formation of the shell and the secretion of the ligament, the organ plays a major part in several other functions. It receives sensory stimuli and conveys them to the nervous system and assists in the shedding and dispersal of eggs during spawning (see ch. XIV). The mantle also participates in respiration by providing direct exchange of gases between the surface tissues of the oyster and the surrounding water. It stores reserve materials (glycogen and lipids), secretes large quantities of mucus and, finally, aids in excretion by discarding blood cells loaded with waste products.

APPEARANCE

The appearance of the mantle reflects the condition of the oyster. At the time of sexual maturity it is a creamy-yellowish color. In oysters which have accumulated large amounts of glycogen with the onset of the cold season the mantle is white and thick. In oysters of poor quality or in those which have not yet recovered after spawning, the mantle is so transparent that the brown or greenish color of the underlying digestive organ is clearly visible through the thin and watery tissue. Oysters in this condition are particularly suitable for the study of muscles, blood vessels, and nerves which in good quality, "fat" oysters are covered by a thick layer of reserve materials.

Pigment cells are concentrated along the free edge of the mantle and in the tentacles in a band varying in color from light brown to jet black. Also, accumulation of copper in the blood cells may produce a distinct green coloration. Different intensities of pigmentation are often found in oysters of identical origin growing together, and cannot be correlated with geographical location or type of bottom.

ANATOMY

For a detailed study of the mantle the oyster should be fully narcotized by Epsom salt (see p. 65) or by refrigerating it overnight at a temperature of about 2° to 4° C. After the valves are forced apart and the body dissected along the median plane, the two halves of the oyster are left attached to their respective valves and the mantle is preserved in its natural position by having a large quantity of fixing fluid poured over it. Portions of the mantle required for study are cut off, stained, dehydrated, cleared, and mounted. In this way very satisfactory whole mounts can be obtained.

The two lobes of the mantle are joined together at the dorsoposterior margin, and form a cap or hood which covers the mouth and the labial palps (fig. 71). Along the anterior and ventral sides of the body the lobes are free and follow the curvature of the shell. When the oyster opens its shell the mantles separate with the valves to which they adhere, leaving a narrow opening between the two lobes through which sea water can enter the mantle cavity. The edge of the mantle, may, however, occupy various positions: it may extend parallel and beyond the edge of the valves to leave a wide space between the two opposing lobes, or it may bend inward almost perpendicular to the shell surface (fig. 74) to reduce or completely close the opening between the two lobes and thereby limit the access of water to the mantle cavity. The behavior of the mantle edge as a regulatory mechanism controlling the flow of water through the mollusk will be discussed later (p. 185). In a closed oyster the mantle edge is located about midway between the distal margin of the gills and the edge of the shell. Its position is marked by an impression called the pallial line, which is less pronounced in the oysters than in clams and some other bivalves.

At the ventroposterior end of the body the two opposing lobes of the mantle join the gills to form the delicate outside wall of the cloaca (figs. 72 and 75, cl., f.). On the left side of the body the mantle is joined to the visceral mass; on the right side it is separated from the visceral mass by the promyal chamber. The fusion of the mantle with the visceral mass and with the bases of gill plates forms the wall of the epibranchial chamber, which leads to the cloaca (fig. 75, cl.). The relative position of the epibranchial and promyal chambers can be seen in the cross section of the oyster made through the dorsal part of the body (fig. 73, ep.br.ch.; pr.ch.).

An oblong slit between the two mantle lobes on the dorsoposterior side of the body marks the opening of the promyal chamber. The inside of this chamber can be examined by completely narcotizing the oyster and forcing its valves apart as far as possible without tearing the adductor muscle. Viewed from the posterior side the promyal chamber in a relaxed oyster appears as an oval cavity (fig. 75) to the left of the adductor

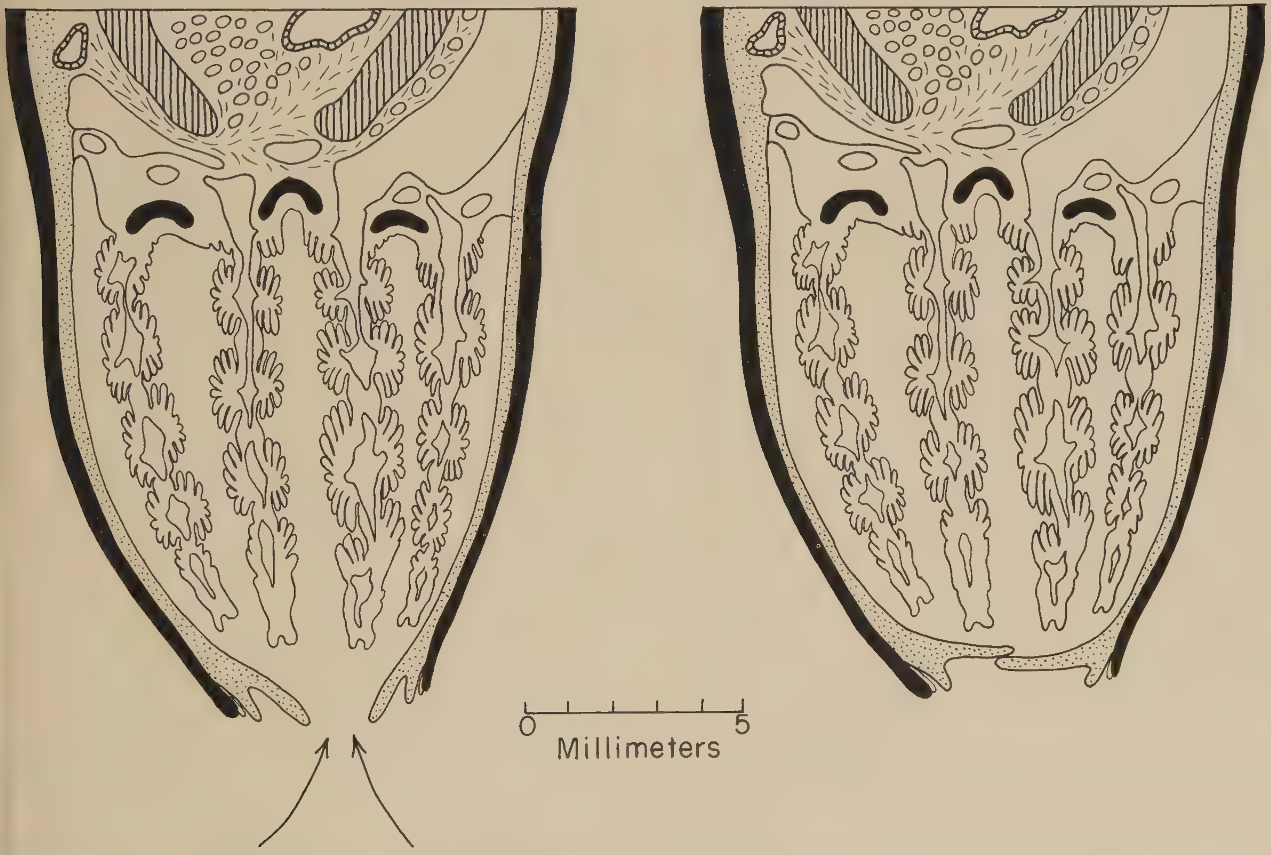

Figure 74.—Cross sections of the valves, mantle, gills, and adjacent portion of the visceral mass of *C. virginica*. In both diagrams the valves are open; the open pallial curtain (at left) permits free access of water to the mantle cavity; the closed pallial curtain (at right) prevents water from entering the mantle cavity. The outer lobe adheres closely to the valve and is not visible. Drawn from the photomicrographs of cross section of adult oyster. Bouin, hematoxylin-eosin.

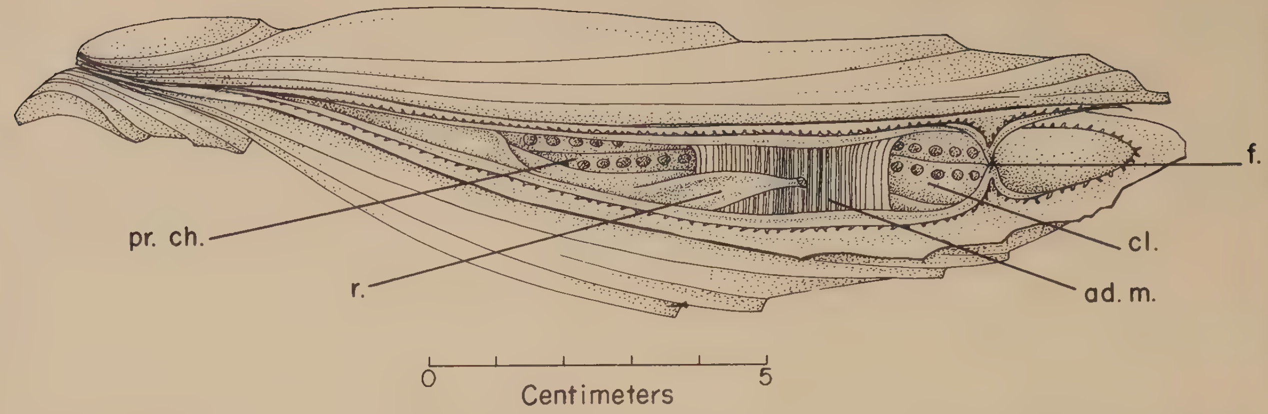

FIGURE 75.—Promyal chamber (at left) and cloaca (at right) viewed from the posterior side of a large oyster (*C. virginica*) completely relaxed by narcosis. Note the fusion of the two opposing lobes of the mantle, and the adductor muscle (in the middle). Drawn from life. Actual size. ad.m.—adductor muscle; cl.—cloaca; f.—fusion of mantle lobes and gills; pr.ch.—promyal chamber; r.—rectum.

muscle. The large round openings of the water tubes of the gills can be seen on the inner wall of the chamber. The rectum extends along the edge of the chamber, ending with a round anus adhering to the side of the adductor muscle; the opening of the cloaca lies to the right of the muscle. The water tubes emptying into the cloaca and the fusion of the mantle with the gill lamellae are also clearly visible.

The most conspicuous components of the mantle are the radial muscles, the blood vessels, and the nerves (fig. 76). All these structures can be identified in a piece of fresh tissue stretched over a glass slide and examined under strong illumination with a low-power microscope. For more detailed study, it is necessary to prepare whole mounts or to section the preserved tissues.

The radial muscles extend from the place of their attachment to the visceral mass to the edge of the mantle. At about two-thirds of their length from their base they begin a fanlike expansion toward the periphery before terminating in the base of the tentacles. The majority of the muscles are accompanied along their length by nerves, blood vessels, and blood sinuses. Much more slender than the radial muscles are the concentric muscular bands which parallel the free edge of the mantle (not shown in fig. 76) and are more abundant at its thickened distal edge.

Because of its strongly developed musculature, the mantle is highly contractile. It may stretch a considerable distance beyond the edge of the valve, or withdraw inside the shell, and even roll up into a tube. Contraction of the radial muscles will throw the inner surface of the mantle into ridges which serve as temporary channels for discarding mucus and foreign particles accumulated on it. These movements may involve either the entire surface of the mantle or only a small portion of it, depending on the intensity of stimulation received by the tentacles.

The wide circumpallial artery (fig. 76, cp.a.) follows the entire periphery of the mantle. At low magnification it is usually visible as a wide tubular structure with many branching vessels which communicate with the irregular spaces (blood sinuses) within the connective tissue. A large pulsating blood vessel, called the accessory heart (ch. XI, fig. 236), is located in the anteroventral part in each lobe of the mantle. The structure and the function of this vessel are discussed in chapter XI, p. 254.

Just outward from the circumpallial artery runs the circumpallial nerve, which also extends along the entire margin of the mantle. In whole mount preparations seen under low power, the circumpallial nerve appears as a compact unbranching band. Examination under high power, however, reveals a fine network of small nerves connecting the circumpallial nerve with nerves and with the visceral and cerebral ganglia. Since nerve fibers on the surface of the mantle and in the tentacles lead to the circumpallial nerve, stimuli received by the neuroreceptors of these areas are transmitted through the circumpallial nerve to the radial nerves and reach either the visceral or the cerebral ganglia.

The thick and muscular border of the mantle is divided into three lobes (fig. 77) which have been described in the literature as "folds" (Awati

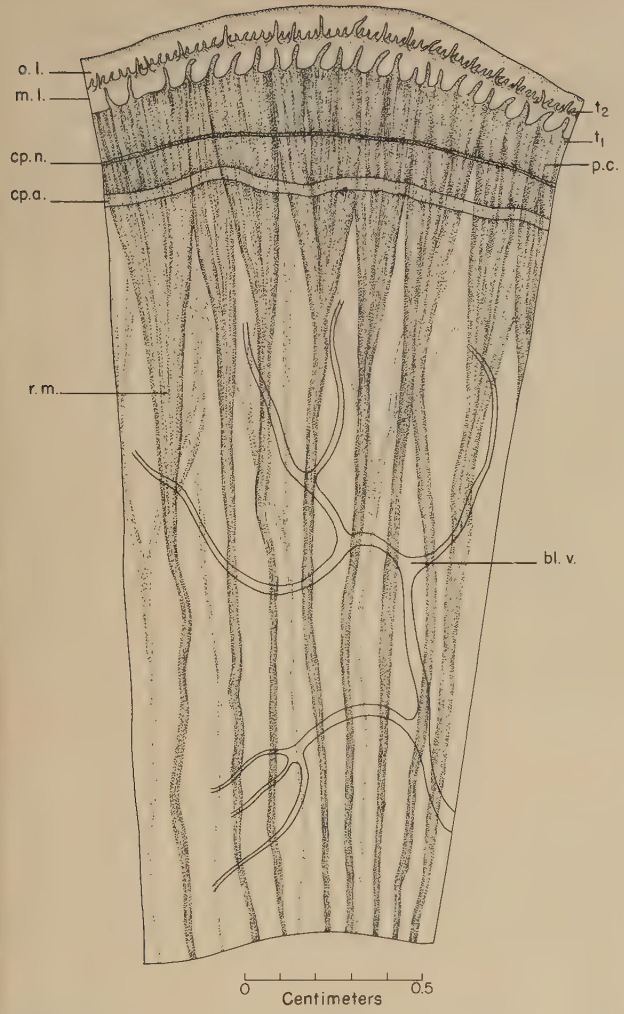

FIGURE 76.—Whole mount of a piece of mantle. Major portion of connective tissue was removed by maceration. Safranin stain. Magnified about 10 times. bl.v.—blood vessels; cp.a.—circumpallial artery; cp.n.— circumpallial nerve; m.l.—middle lobe; p.c.—pallial curtain (inner lobe); r.m.—radial muscle; o.l.—outer lobe or shell lobe; t_1—tentacles of inner lobe; t_2—tentacles of middle lobe. Radial nerves surrounded by radial muscles are not visible. Formalin 5 percent, hematoxylin.

and Rai, 1931), "reduplications" (Nelson, 1938; Pelseneer, 1906), "lamellae" (Hopkins, 1933), "lames" in French (Leenhardt, 1926), and "Klappe", in German (Rawitz, 1888). The term "reduplication" is misleading because the lobes are not formed by the duplication of the mantle tissue, being comparable rather to a fringe or flounce at the margin of a soft material. To avoid confusion the term marginal lobes is retained in this text.

The mantle border of all the species of oysters studied, namely, *C. virginica*, *C. angulata*, *C. gigas*, *O. edulis*, and *O. lurida* is divided into three projecting lobes, the outer or shell lobe (sh.l.), the middle lobe (m.l.), and the inner lobe or pallial curtain (p.c.). Hopkins' statement (1933, p. 483) that "The border of the mantle (of *C. gigas*) divides into two lamellae, each bearing a row of tentacles" is an obvious inaccuracy of description.

The outer or shell lobe (sh.l.) is narrow and devoid of tentacles. It lies in contact with the margin of the shell and may be seen protruding beyond the edge of the valve during periods of rapid growth. The middle and the inner lobes each bear a row of sensitive and highly contractile tentacles.

The inner lobe or pallial curtain (fig. 77, p.c.) is especially broad and turned inward. In describing this structure in scallops Pelseneer named it the "velum" (1906). Although that term has been used by several investigators (Awati and Rai, 1931; Dakin, 1909b) Nelson (1938) pointed out that the term "velum" is better known as the swimming organ of the pelecypod larvae and proposed to call the inner lobes of the mantle the "pallial curtains". This term seems to be appropriate, but is used in this book in the singular since there appears to be no advantage in the plural recommended by Nelson.

The inner lobe may be projected into the mantle cavity (fig. 74). Depending on the degree of contraction of various sets of muscles the inner lobe assumes different angles in relation to the mantle as a whole. In a fully relaxed mollusk the lobe of each side extends outward in the general plane of the mantle and shell. In a contracted state the lobes on both sides project inward almost at right angles to the surface of the mantle; in this position the mantle borders touch and the tentacles of the two sides interlock, effectively sealing the entrance to the mantle cavity. This function of the inner lobe was first described by Rawitz in 1888 and was redescribed in 1933 by Hopkins. As will be shown later (p. 304) the pallial curtain also plays an important role during the spawning of female oysters.

The deep furrow between the shell lobe and the middle lobe is called the periostracal groove (fig. 77, per.gr.), the name referring to the secretion site of organic shell material by glandular cells concentrated in the deepest portion of the groove and collectively known as the periostracal or conchiolin gland (c.gl). During the shell-

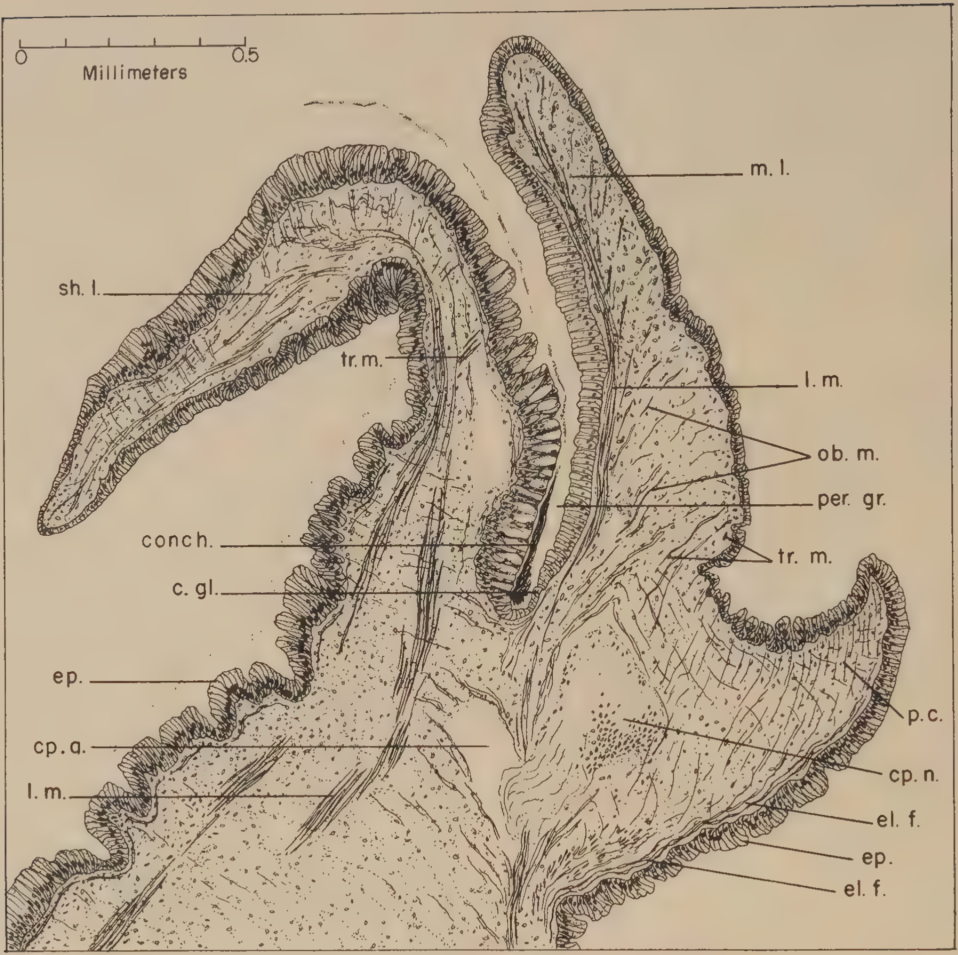

FIGURE 77.—Transverse section of the edge of the mantle of adult *C. virginica*. Bouin 3, hematoxylin-eosin. The outer or shell lobe at left faces the valve (not shown) and is bent as a result of fixation. The section passes between the tentacles of the inner lobe (pallial curtain); only the tentacle of the middle lobe (m.l.) is seen. c.gl.—conchiolin (or periostracal) gland; conch.—sheet of conchiolin spread over the shell lobe; cp.a.—circumpallial artery; cp.n.—circumpallial nerve; el.f.—elastic fibers; ep.—epithelium; l.m.—longitudinal muscles of tentacles; m.l.—middle lobe; ob.m.—oblique muscles; p.c.—pallial curtain (inner lobe); per. gr.—periostracal groove; sh.l.—shell or outer lobe; tr.m.—transverse muscles.

growing season, viscous yellowish material (fig. 77, conch.) accumulates in the groove and gradually oozes out to the periphery of the outer mantle lobe, where it solidifies into the periostracum. The groove between the middle lobe (m.l.) and the pallial curtain secretes mucus, which is gradually moved by ciliary currents to the outer margin of the mantle and there discarded.

It has already been noted that the edges of the middle and the inner lobe each bear a row of highly extensible, tapering tentacles; however, their arrangement and size in the two lobes are different. Two types are clearly visible along the edge of the middle lobe: numerous short and slender tentacles, and less abundant long and stout ones (fig. 76). The order of the tentacles follows a certain pattern, namely, each long tentacle is succeeded by a group of four to six small ones (t_2). The stout tentacles frequently occupy a position slightly out of line with the small ones, being a little nearer to the inner fold. The inner lobe bears only the long and stout tentacles (t_1).

There is great variation in the size of all the tentacles and in their pigmentation. Since they are highly sensitive to touch and other stimuli and retract at the slightest disturbance, their relative size can be observed only when they are completely relaxed. In fully narcotized adult oysters the ratio between the numbers of tentacles on the inner and middle lobes was found to vary from 10:18 to 10:32.

It has not yet been definitely established whether the two types of tentacles contain different receptors and therefore respond to different stimuli. According to Elsey (1935) the large tentacles of *C. gigas* are more sensitive to hydrochloric acid than the small ones. Hopkins (1932) does not specify which row of tentacles was under observation in his work on sensory stimulation of *C. virginica*. In my experiments (see p. 293) observations were made exclusively on the long tentacles of the inner lobe.

A narrow and slightly pigmented cylindrical structure along the dorsal edge of the mantle (fig. 78) marks the position of the subligamental ridge, the organ which secretes the ligament. The ridge consists of a layer of specialized epithelium underlined by connective tissue. Large blood vessels are found close to the base of the ridge. Microscopic structure of the ridge is given on p. 83.

RUDIMENTARY MUSCLE OF THE MANTLE

A small and sometimes hardly visible muscle is located on the dorsal part of the mantle. Its location is sometimes marked by light violet pigmentation and by a shallow depression in the corresponding part of the valve to which the muscle adheres. The attachment is weak, and in the majority of oysters the muscle separates from the valve when the valve is lifted. Leenhardt (1926) states, however, that in some *O. edulis* the muscles were so strongly attached to the shell that they could not be separated without rupturing the mantle tissue. Examination of sections of the mantle of *C. virginica* from the Woods Hole area convinced me that muscle fibers do not extend from one side to the other, but end in the connective tissue of the mantle. The muscle is apparently nonfunctional and morphologically is not analogous to the anterior adductor of bivalves. Leenhardt (1926) considers the rudimentary muscle of the mantle as a vestige of the larval foot retractor which disappears during metamorphosis. Stenzel (1963) states that this muscle is present in all the Ostreidae and calls it Quenstedt's muscle in honor of its discoverer (Quenstedt, 1867).

HISTOLOGY

The mantle consists of connective tissue which envelops the muscles, blood vessels, and nerves and is covered on both sides with the epithelium.

CONNECTIVE TISSUE

The most conspicuous structural element of the connective tissue is the vesicular cell, characterized by large globular or oval body and relatively small nucleus without nucleoli. In zoological literature these cells appear under a variety of names and were even incorrectly considered as lacunae (Leenhardt, 1926) and mucus cells (List, 1902). Well-developed membranes outline cell boundaries sharply; the protoplasm within forms a delicate network of fine granules. In preparations dehydrated with alcohol the inside of the vesicular cells appears almost empty, but in tissues treated with osmic acid and in frozen sections stained with Sudan II and other fat stains large globules of lipids are seen to fill the inside of the cells (figs. 79 and 80). Less abundant are the smaller round cells with more compact protoplasm. They often occur near small arteries (fig. 81, r.c.). The fusiform cells (f.c.) with small bodies and oval nuclei form long branching processes which anastomose and touch each other.

Examination of frozen sections of connective tissue treated with toluidine blue or other metachromatic stains shows clearly the presence of a cytoplasmic ground substance with a very fine reticulum supporting various inclusions. After the removal of glycogen this substance can be stained very deeply with periodic acid fuchsin (McMannus reagent)or with Hale stain which is used to test for acid polysaccharides of the hyaluronic acid type (Hale, 1946). The results of such staining reactions have been interpreted in the literature as indicating the presence of mucopolysaccharides or mucoproteins. Histological methods are not entirely dependable (Meyer, 1957), but so far no chemical analyses of the connective tissue of the mantle have been made. It is known, however, that acid mucopolysaccharides are among the components of the ground substances in mammalian tissues. It is very likely that they are also present in the connective tissue of the oyster.

Elastic fibrils are scattered throughout the connective tissue of the entire thickness of the mantle but appear to be more abundant at the free edge and in the layers underlying the surface epithelium (fig. 77, el.f.). Muscle fibers are also very abundant and will be discussed in detail later.

In some specimens the mantle may be thin and transparent whereas in others it is thick and

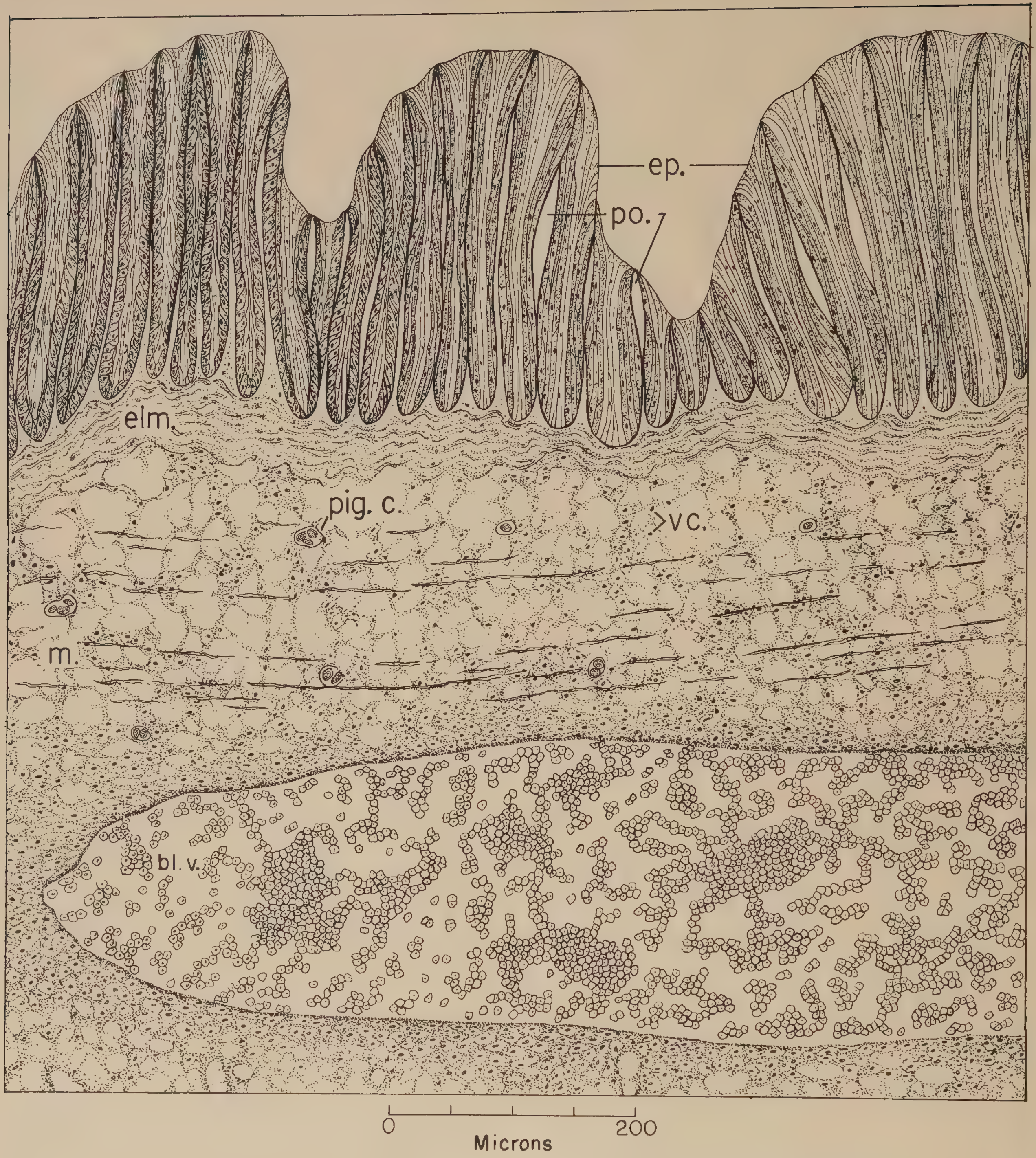

FIGURE 78.—Longitudinal section of the subligamental ridge made at right angles to its dorsal surface. Bouin 3, hematoxylin-eosin. bl.v.—large blood vessel; el.m.—basal elastic membrane; ep.—epithelium; m.—muscle fibers; pig.c.—pigment cells; po.—pockets between the epithelial cells; v.c.—vesicular cells.

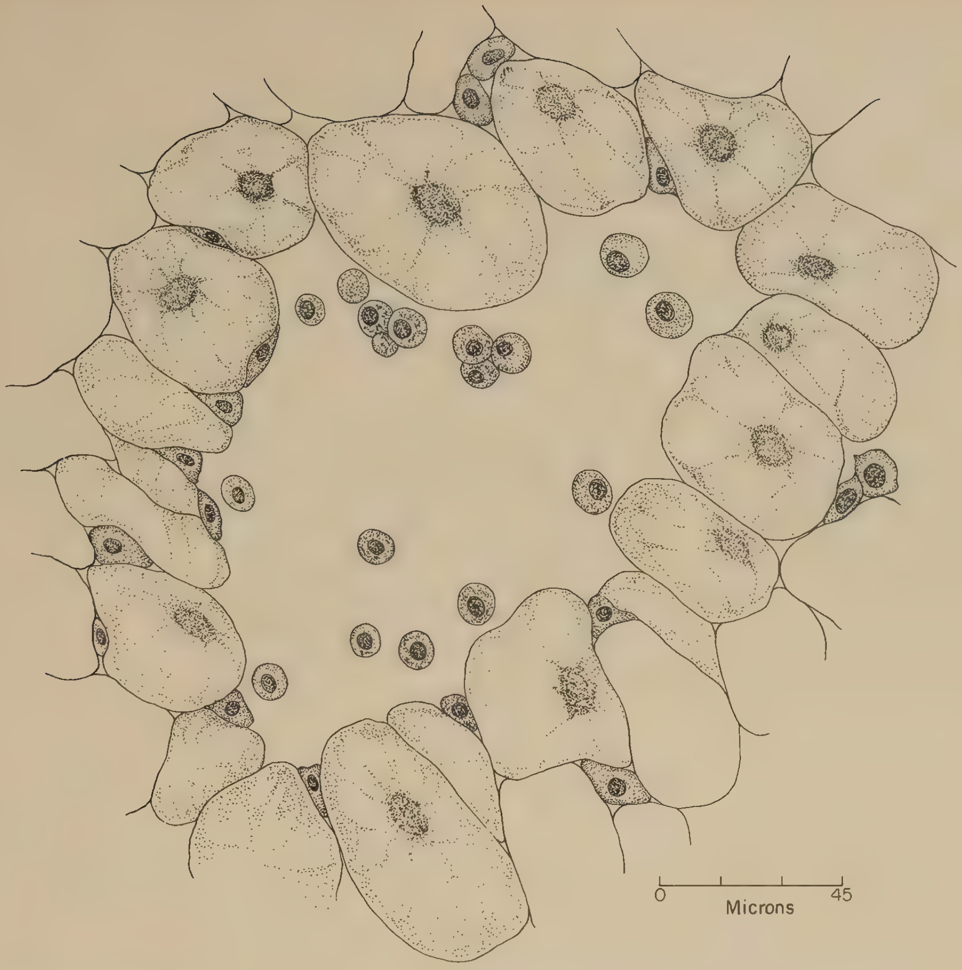

FIGURE 79.—Vesicular cells of connective tissue from the mantle of an adult *C. virginica* surrounding the blood sinus. Blood cells crawl between the cells of connective tissue and penetrate into the sinus. Bouin 3, hematoxylin-eosin.

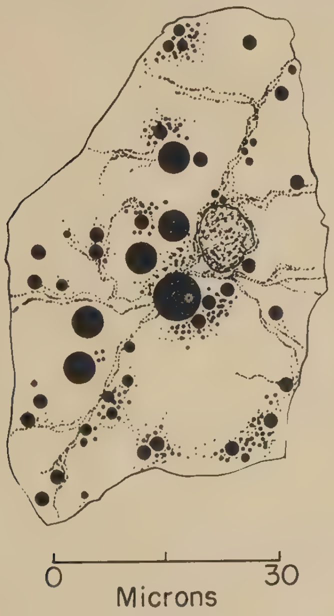

FIGURE 80.—Vesicular cell of connective tissue with fat globules. Frozen section. Sudan II.

opaque. These changes in appearance usually coincide with seasonal cycles in the glycogen content of the connective tissue and with the progressive stages of gonad development.

The presence of glycogen can be easily demonstrated by treating the tissue with Lugol solution (1 percent iodine in 2 percent potassium iodide in water). Specific reagents used for the identification of glycogen, such as Best's carmine and Bensley's modification of Bauer-Feulgen reagent (which stains glycogen granules red-violet), also give good results.

In the live oyster glycogen can be seen as small colloidal granules which ooze from the tissue under slight pressure. In preserved and stained material it appears in the form of granules or rods (fig. 82). The total amount of glycogen in the connective tissue may be so great that the blood vessels and nerves of the mantle are completely hidden under it and cannot be traced by

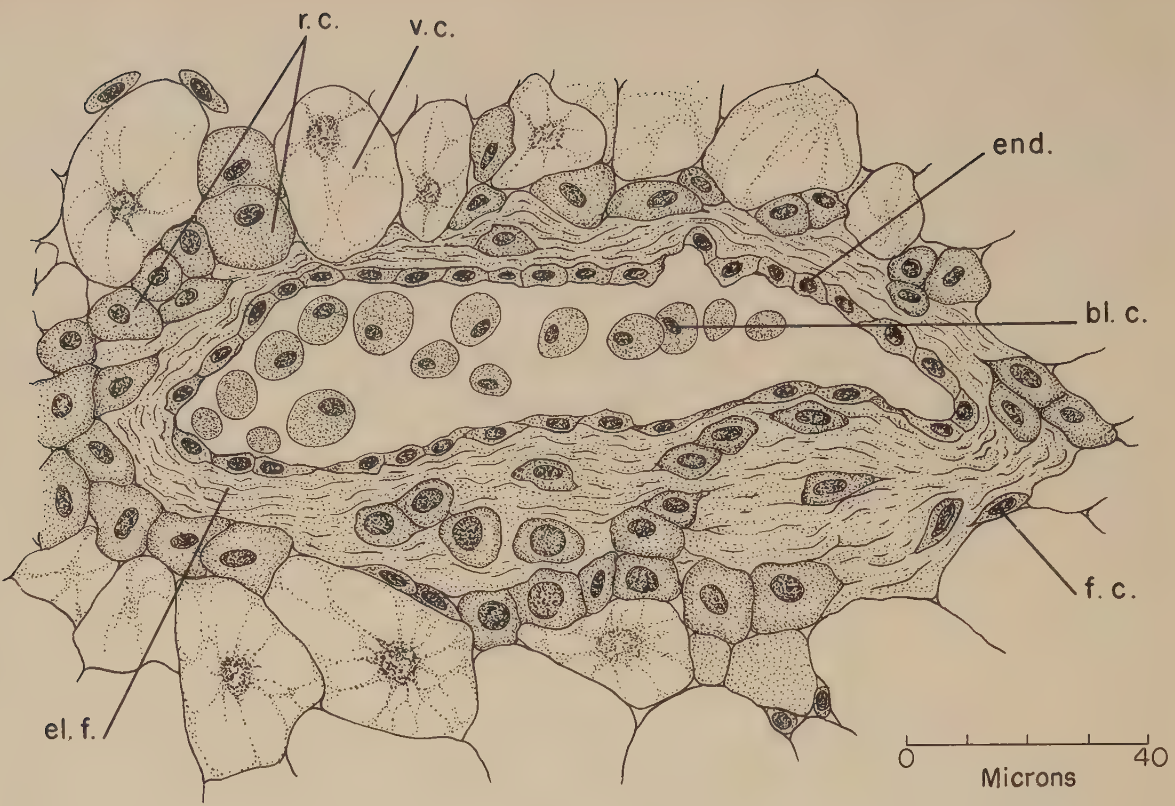

Figure 81.—Cross section of a small artery of the mantle. bl.c.—blood cells; e.lf.—elastic fibrils; end.—endothelium; f.c.—fusiform cells; r.c.—round cells; v.c.—vesicular cells. Kahle, hematoxylin-eosin.

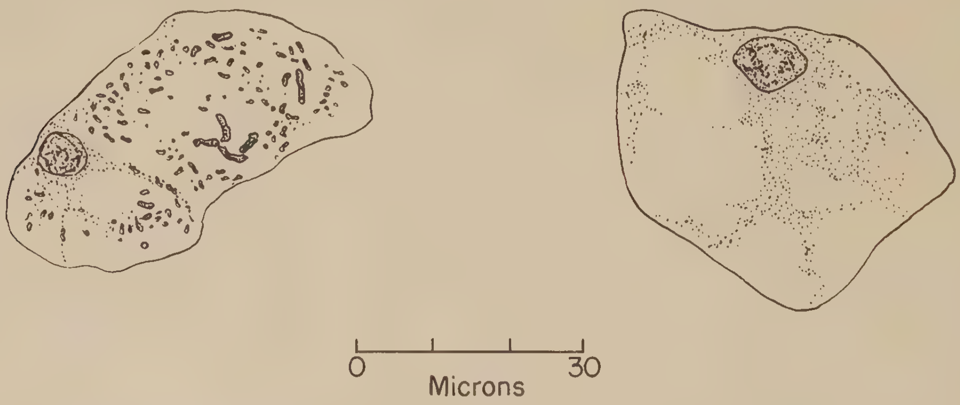

Figure 82.—Two vesicular cells from the mantel of an adult *C. virginica*. Left—the cell contains glycogen stained with Best's carmine; fat globules were dissolved in processing. Right—similar cell after fixation with Bouin 3; note complete absence of glycogen and fat, both dissolved during fixation and dehydration.

dissection. Such abundance of reserve material led one of the earlier investigators (Creighton, 1896, 1899) to conclude that its storage in the connective tissue of lamellibranchs is a special adaptation comparable to the storage of fat in the connective tissues of vertebrates.

The quantity of glycogen stored in connective tissue gradually decreases as the gonads of the oyster increase in bulk. This was first reported for *O. edulis* by Pekelharing (1901) and confirmed by the more recent investigations of Bargeton (1942). Evidence presented in the latter work strongly suggests that the growing sex cells utilize the glycogen stored in the vesicular cells surrounding the gonad tubules, but cytological details of this process are still unknown and the problem has not yet been studied from a biochemical point of view.

After the disappearance of their contained glycogen the vesicular cells do not shrink or collapse. A hypothesis was therefore advanced (Semichon, 1932) that the glycogen granules are supported by a framework of a special substance which remains intact after the dissolution of glycogen. It is claimed that this framework can be revealed by staining with black anilin inks. The evidence for the existence of such a special substance is not, however, convincing. In cells with a moderate content of glycogen the latter can be seen in close contact with the protoplasmic network typical for vesicular cells. Furthermore, the walls of the vesicular cells are fairly rigid and the cells retain their shape even when they are empty. The shrinkage of connective tissue frequently caused by changes in osmotic pressure when the salinity of the water surrounding the oyster is suddenly increased is not associated with the disappearance of glycogen.

The fat globules in vesicular cells vary greatly in size and number, usually forming distinct vacuoles that are easily dislodged. The relationship between the fat and glycogen content of the oyster and the role of lipids in the physiology of lamellibranchs have not been studied.

Large oval cells containing a brown pigment are scattered throughout the connective tissue of the mantle. The pigment is not soluble either in acids or fat solvents. Its chemical nature and physiological significance are not known.

Wandering blood cells are commonly seen in the mantle. They crawl between the connective tissue cells, aggregate in the vicinity of blood vessels and blood sinuses (fig. 79), and are gradually discarded through the surface of the mantle. As a rule, the oyster continually loses a certain amount of blood by diapedesis or bleeding. Any excess of heavy metals accumulated by blood cells (see p. 390) is also discarded by this normal process.

MUSCLES

The radial muscles consist of large, regularly spaced bands of fibers which extend almost the entire width of the mantle from the line of its fusion with the visceral mass and with the adductor muscle to the free margin. For a study of the anatomy of the muscular system the connective tissue in which the bands are firmly enclosed should be macerated in 1 percent potassium hydroxide for about 24 hours. After being washed in distilled water the loosened tissues are removed with a small stiff brush and fine forceps.

The radial muscle bands are composed of large bundles of unstriated fibers which begin to branch toward the distal edge of the mantle about one-third of the distance from that edge. At this level the muscles appear fanlike and enter into all three lobes, where they terminate.

The central part of a muscle band is usually occupied by one or two radial nerves, although muscles without a central nerve (figs. 83 and 84) do occur.

The contraction of the radial muscles pulls the entire mantle inside and throws its surface into ridges. Such a general reaction usually precedes the contraction of the adductor muscle and the closing of the valves. The contraction may occur spontaneously in response to some internal stimulus or it may develop as a result of external irritation produced by chemicals, mechanical and electrical shock, or sudden change in illumination. In response to a weak outside stimulus only a small sector of the mantle contracts, making a slight V-shaped indentation along its periphery. This response may or may not be followed by contraction of the adductor muscle. Strong stimuli, as a rule, result in complete withdrawal of the mantle, contraction of the adductor muscle, and closing of the valves. Besides the large radial bands there are many smaller bundles of transverse fibers (fig. 77, tr.m.) extending diagonally across the thickness of the mantle, a well-developed system of longitudinal muscles (l.m.), and the oblique muscles (ob.m.) of the tentacles.

The longitudinal or concentric muscles follow the general outlines of the edge. They are more abundant at the thickened distal edge of the mantle but do not exhibit the definite pattern of distribution apparent in the radial muscles. The transverse muscle fibers are more numerous in the pallial curtain (fig. 77, tr.m.) than in the other parts of the mantle. They are so arranged that the position of the curtain may be quickly changed in response to external or internal stimuli.

All the muscle cells are of the smooth, nonstriated type with typical elongated nuclei. In some bivalves the muscle fibers of the mantle appear to show a double oblique striation; this was shown to be an optical effect created by a series of fine fibrillae spiralling around the larger fibers (Fol, 1888; Marceau, 1904). Muscle fibers

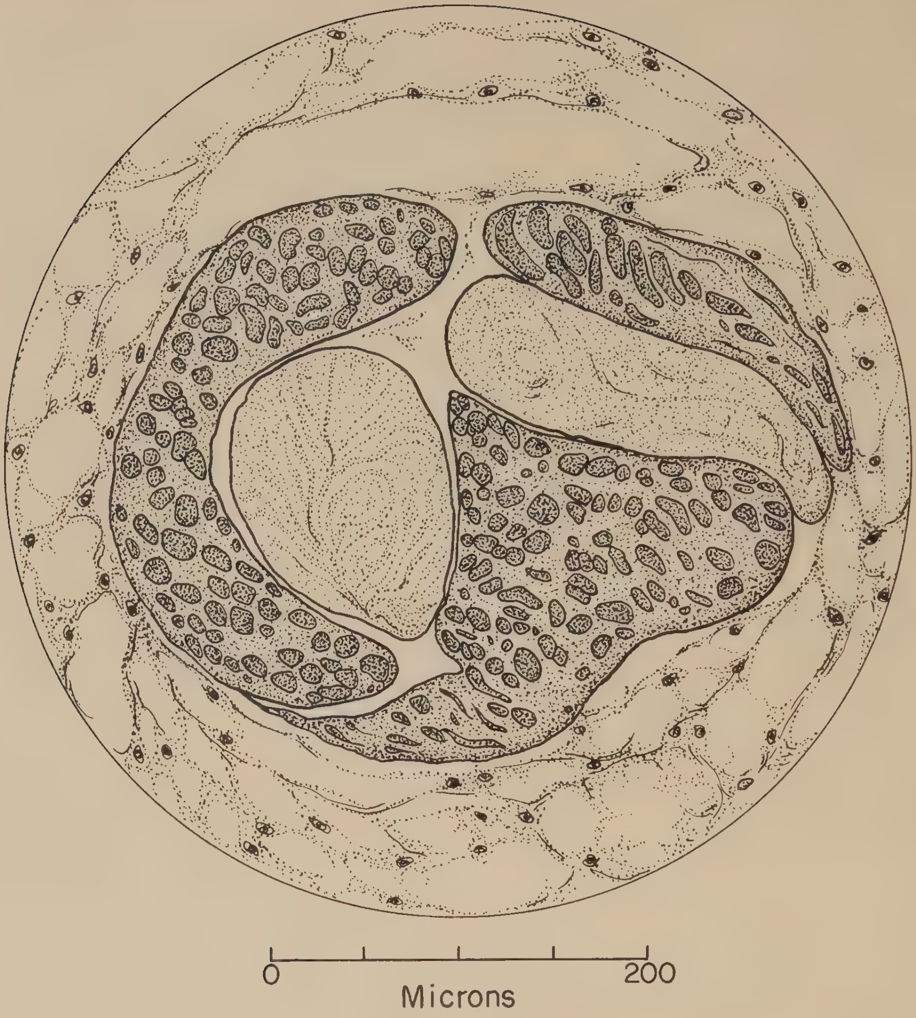

FIGURE 83.—Cross section of the radial muscle of the mantle of an adult *C. virginica*. The muscle completely surrounds two nerves. Bouin 3, hematoxylin-eosin.

with true transverse striation, described in the mantle of *Pecten jacobaeus* and *P. opercularis* (Dakin, 1909a), are not found in the oyster mantle.

BLOOD VESSELS

The principal blood vessels of the mantle (fig. 232 in ch. XI) are the circumpallial artery (cr.p.a.), which runs along its entire periphery and sends out many branches; the common pallial artery (co.p.a.); and a large pulsating vessel in the anteroventral part of the mantle called the accessory heart (fig. 236 in ch. XI). The latter can be observed by dissecting the wall of the epibranchial chamber and spreading the cut tissues apart. The structure and function of these vessels are discussed on page 253.

The small arteries and veins of the mantle can be recognized easily by their histological characteristics. The walls of the arteries have a thick, elastic, muscular layer lined with endothelium (fig. 81, end.). In the veins the elastic layer is much less developed and the endothelium absent (fig. 85). The sinuses (fig. 79) are irregularly shaped spaces in the connective tissue. Since they have no walls of their own they cannot contract. The size of the opening or lumen may be reduced by growth of the surrounding vesicular cells and by accumulation of blood cells.

EPITHELIUM, TENTACLES, AND NERVES

Both sides of the mantle are covered by cylindrical epithelial cells set on an elastic basal membrane (fig. 77). Large goblet cells which secrete mucus and cells containing eosinophile granules are abundant on both sides of the mantle. The cells of the side facing the pallial cavity are long

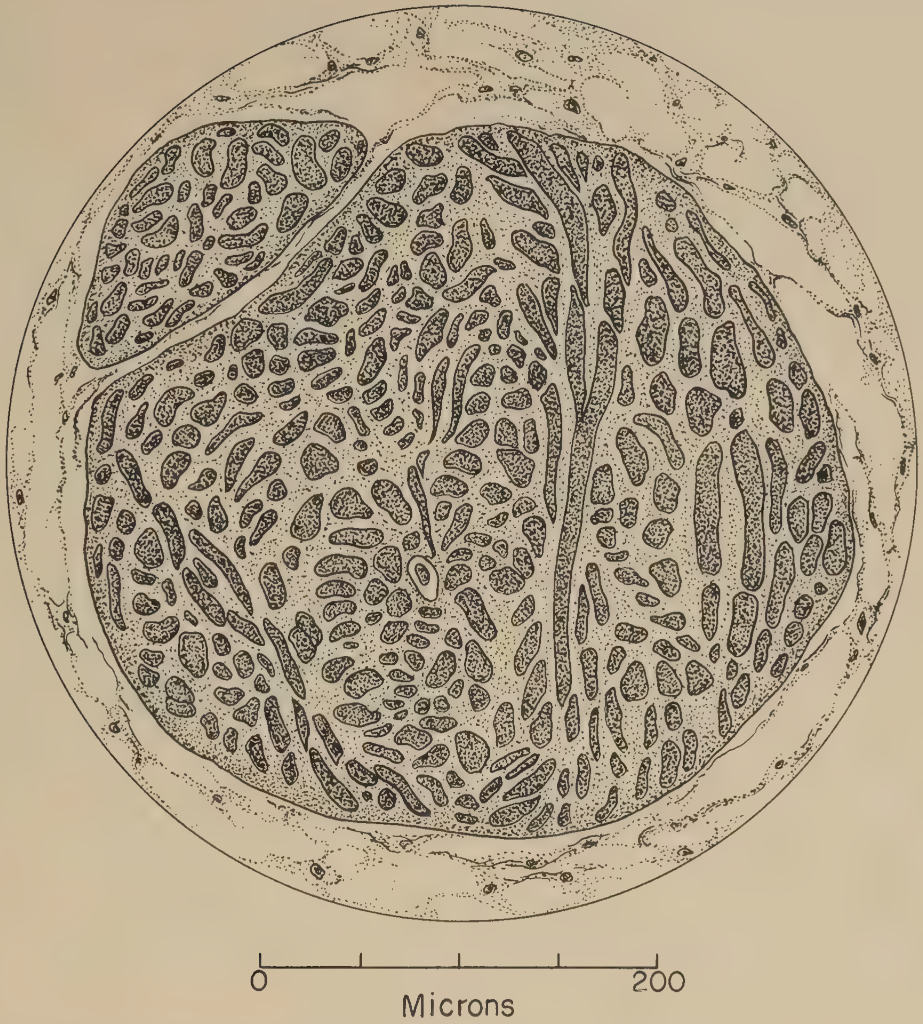

FIGURE 84. Cross section of the radial muscle of an adult *C. virginica*. The muscle is not accompanied by nerve. Bouin 3, hematoxylin-eosin.

and ciliated; those on the outside under the valves bear no cilia and are much shorter, in places almost cubical.

The two sides of the mantle perform different functions. The inner side maintains ciliary currents, which in general move from the base of the mantle to its edge and carry mucus and sediments settled from the water; this material is passed to the margin of the shell to be discharged. The epithelium of the outer side secretes the inner layer of the shell, the so-called calcito-ostracum.

Although the ciliated epithelium of the edge of the mantle contains the same kind and proportion of cellular elements found in other parts of the organ, the cilia at the border of the mantle are especially powerful. The tentacles themselves consist of a core of connective tissue with associated blood vessels, elastic fibrils, and muscle fibers which emerge from branches of the radial muscles. On the outside the tentacles are covered with a single layer of ciliated epithelium to which black or brown pigment imparts a dark color. Special sense organs are absent but the tentacles, especially the long ones, are well supplied with nerves branching out from the nerve which enters the base of the tentacle and is itself connected with the nervous system of the mantle (fig. 86).

The circumpallial nerve provides communication between the tentacles and the radial nerves. The structure of this nerve resembles that of a ganglion: numerous nerve cells of the types found in visceral and other ganglia (see p. 288) occupy the periphery of the nerve; its center consists of nerve bundles with occasional small ganglion cells.

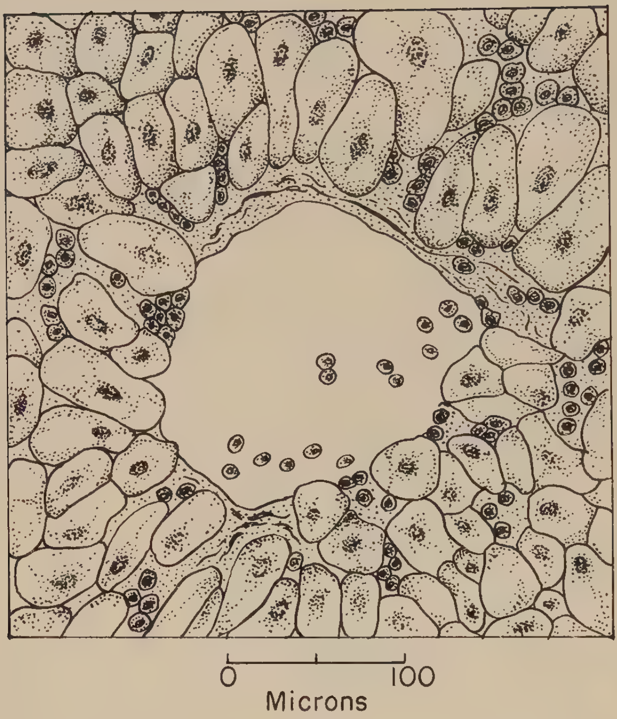

Figure 85.—Transverse section of a small vein of the mantle. Note the absence of endothelium and poorly developed elastic layer. Bouin, hematoxylin-eosin.

Close nerve contact between the muscles and other organs of the mantle is maintained through a fine nerve network which can be made visible by using the gold impregnation method (fig. 87). I have had no success in revealing it with vital stains.

PERIOSTRACAL GROOVE AND GLAND

The narrow space between the middle and the outer lobes of the mantle edge, called the periostracal groove (fig. 77, per.gr.), is lined with ciliated epithelium which is replaced at the bottom of the groove by glandular cells. The innermost part of the groove is called the periostracal gland (fig. 88), although it would have been more appropriate to refer to it not as a gland but as a secretory epithelial surface (Maximow and Bloom, 1930). This surface is covered with a single layer of glandular cells different in appearance and structure from the epithelial cells of the distal part of the groove. Unlike a true gland, it does not form a compact body extending under the surface of the groove and it has no duct. On transverse sections of the mantle edge the gland sometimes appears as a round structure surrounded by connective tissue. Examination of a series of sections shows, however, that this appearance is caused by the invaginations of the inner surface of the lobe. The periostracal gland is present in all lamellibranchs and was the object of many histological studies (Leenhardt, 1926; List, 1902; Moynier de Villepoix, 1895; Rassbach, 1912; Rawitz, 1888).

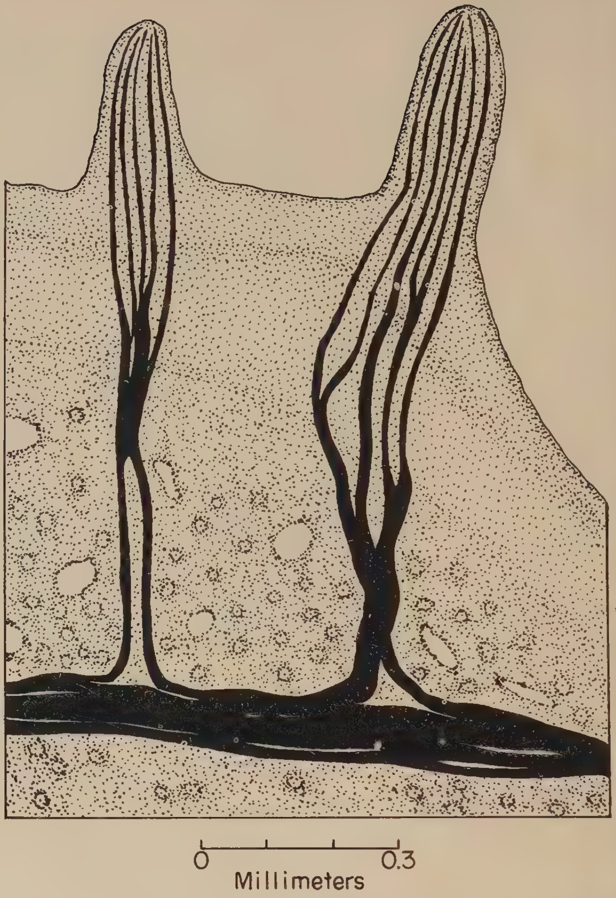

Figure 86.—Innervation of the tentacles of the middle lobe. Formalin 5 percent, gold impregnation. Whole mount.

There is a conspicuous difference in the appearance of the cells along the two sides of the groove. Those lining the outer lobe (fig. 88, left side) are distended at the distal ends and taper toward the base into slender rootlike processes which, according to Rawitz (1888) who described them in the oyster, penetrate the underlying connective tissue. I was not able to reveal such rootlets in my material. None of these cells bear cilia, although the distal part of the groove, not shown in figure 88, is lined with ciliated epithelium. Typical goblet cells containing eosinophile granules, amoebocytes, and round mucus cells are present in the epithelial layer of both sides of the groove.

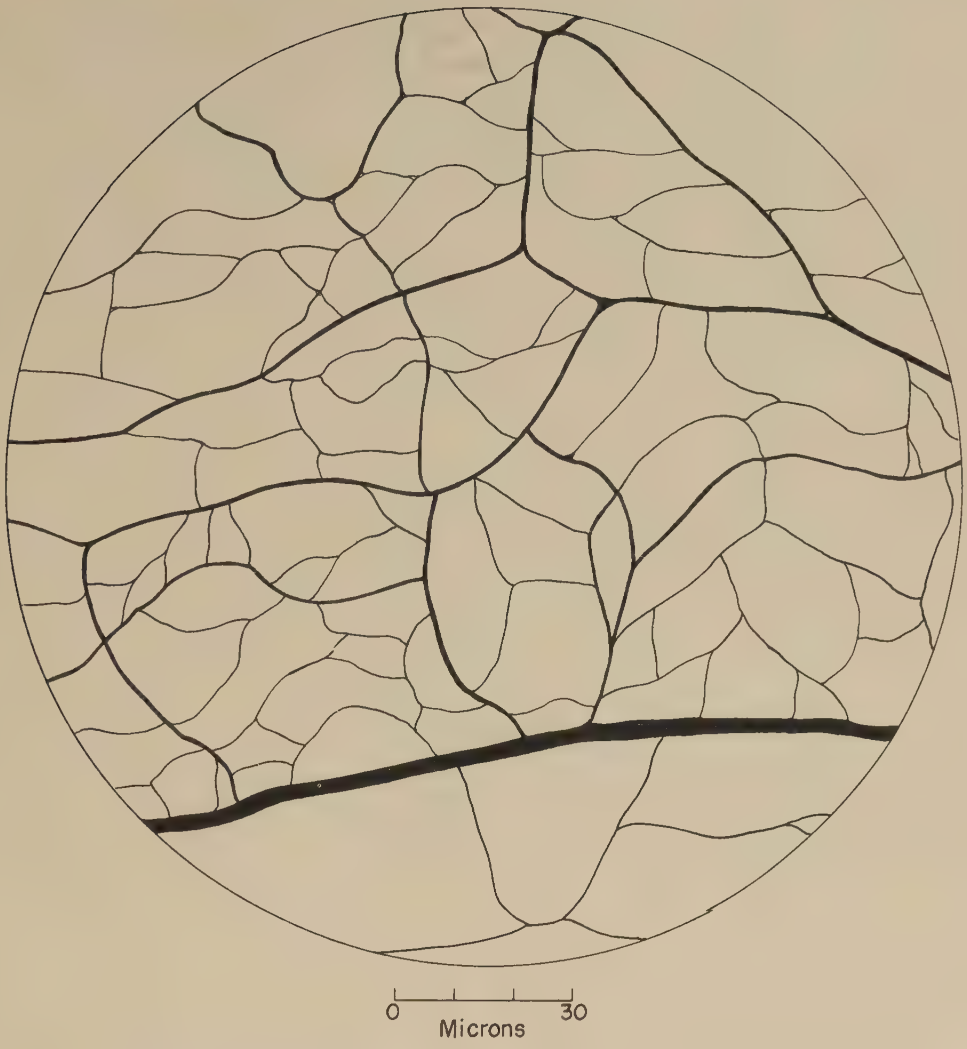

FIGURE 87.—Small area of mantle showing nerve net. Formalin 5 percent, gold impregnation. Whole mount. Camera lucida drawing.

At the very bottom of the groove the tall epithelial cells are suddenly replaced by short cubical cells (fig. 88, right side) which extend a short distance along the inner side of the groove.

The material secreted by the periostracal gland accumulates at the bottom of the groove and in the majority of my preparations appears to adhere to the cells of the outer side (left side of figure 88). This, however, is the result of shrinkage caused by dehydration during the processing of slides. In preparations mounted in glycerin the conchiolin can be seen in close contact with the epithelium of both sides of the groove.

The function of the periostracal gland is to supply large quantities of the material required for new shell growth at the edge of the valves. The organic matrix (conchiolin) and foliated layers of calcite needed for increasing thickness of the valves, on the other hand, are secreted by the epithelium covering the entire outer surface of the mantle and in close contact with the inner surface of the valve. The epithelium consists of nonciliated cells which are cylindrical near the free margin of the mantle but become flattened and almost cubical in more proximal areas. Both conchiolin-secreting and calcium-secreting cells are present in this epithelium but their cytological differentiation by means of staining reactions or by precipitation of calcium oxalate is not reliable. Mucus cells and oval cells containing eosinophile granules also occur throughout the entire surface of the epithelial covering.

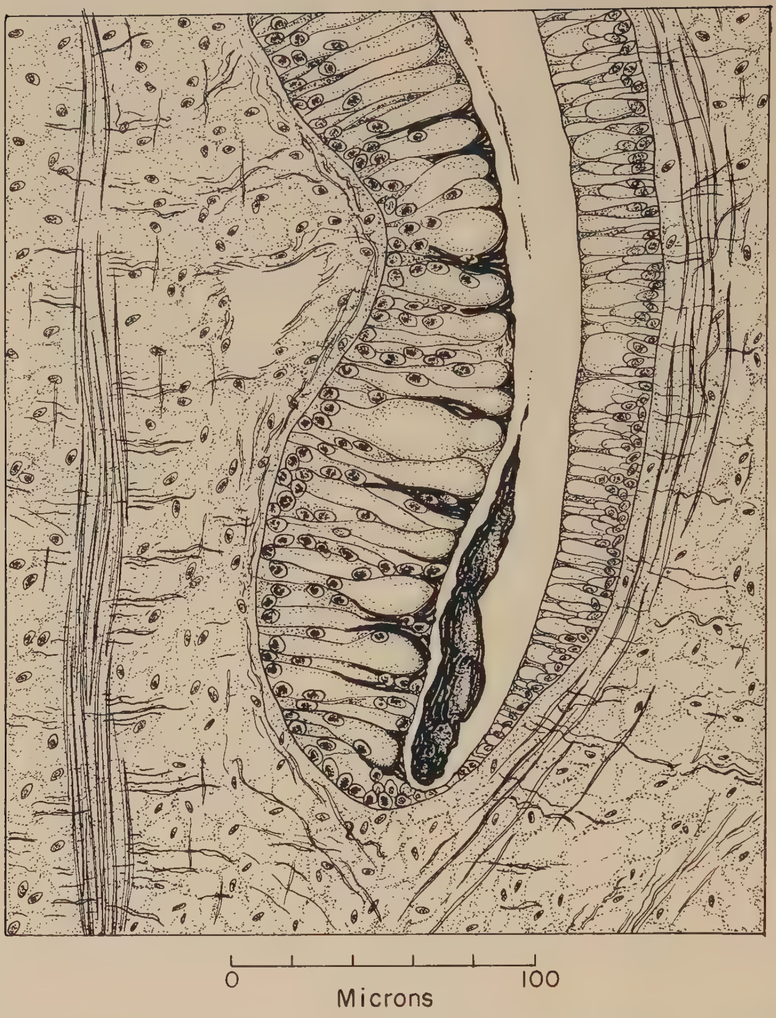

FIGURE 88.—Transverse section of the periostracal groove. The cells on the left side are distended with secretion; those at the very bottom are short, almost cuboid. The black mass at the bottom of the groove is conchiolin. Bouin, hematoxylin-eosin.

Owing to the presence of the conchiolin-secreting cells, the entire outer surface of the mantle is sticky and adheres closely to the inner surface of the shell. List (1902) advanced a theory, not well supported by observation, that in the Mytilidae the mantle adheres to the shell by means of fibrillae which originate in the myoepithelial cells and pass through the epithelium. Such an arrangement is not found in *C. virginica* or in *C. angulata*, and according to Leenhardt (1926) does not exist in *Mytilus*.

The epithelial layer along both surfaces of the mantle including its free edge contains alkaline phosphatase, an enzyme involved in the calcification of the shell. The presence of the enzyme can be demonstrated by the Gomori method (Gomori, 1939, 1943) based on the formation of insoluble calcium phosphate as a result of phosphatase action on sodium glycero-phosphate and calcium ions. Further treatment with 5 percent silver nitrate (or with cobalt nitrate) converts the calcium phosphate to silver (or cobalt) phosphate which turns black after exposure to light. Both reagents gave satisfactory results in demonstrating the localization of the enzyme in the epithelium of the mantle. The strongest reaction, judged by the opacity and width of the black layer, was found to occur along the edges of the mantle and in the area of the periostracal groove. Even the tips of the tentacles contained noticeable amounts of the enzyme (fig. 89). These obser-

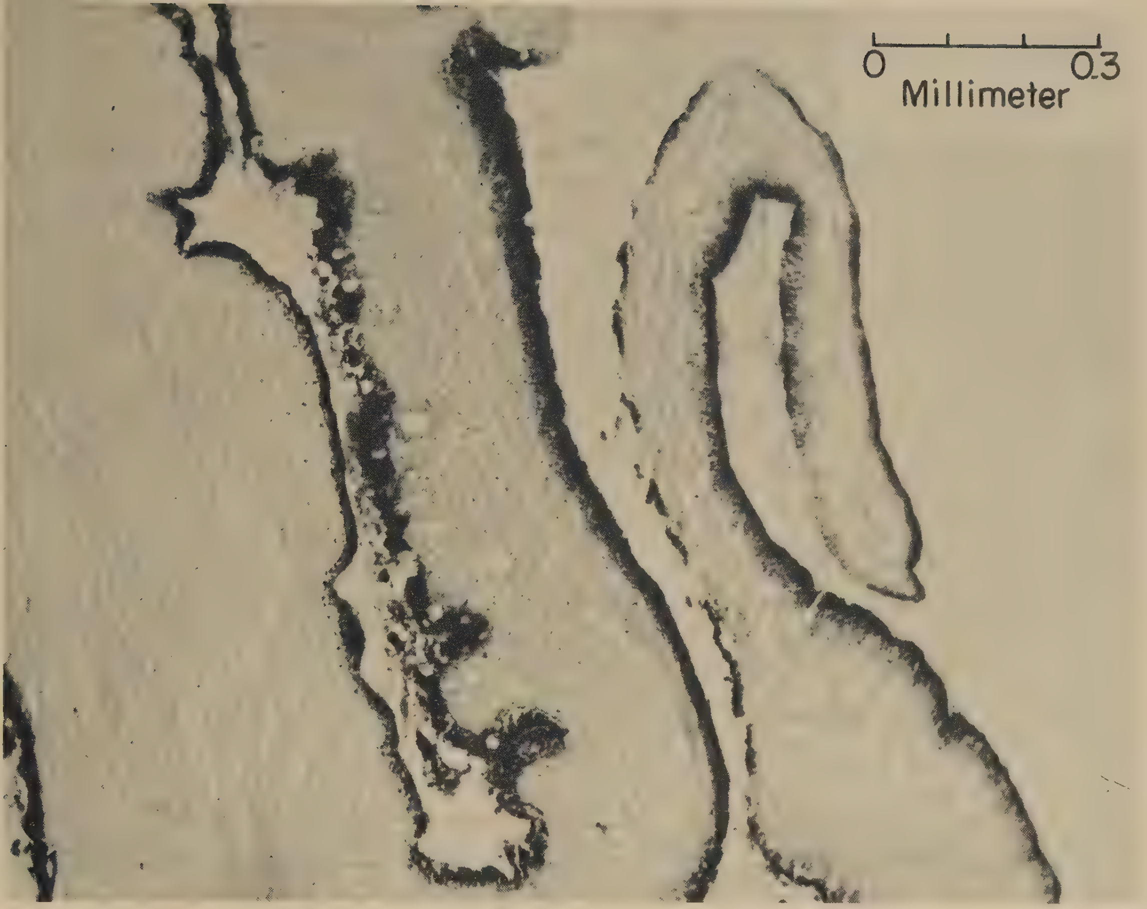

FIGURE 89.—Localization of alkaline phosphatase in the mantle of *C. virginica* (longitudinal section). Photomicrograph of a preparation treated by Gomori method.

vations are in full agreement with the results obtained by Bevelander (1952).

SUBLIGAMENTAL RIDGE

A small ridge marking the dorsal edge of the mantle along the fusion of its two lobes is known as the subligamental ridge. Its length in the anteroposterior direction corresponds exactly to that of the ligament, which is secreted by the epithelial cells of the ridge. The base of the ridge is flattened and rests on basic elastic membrane; the body of the ridge is semicylindrical in cross section, its surface slightly undulating, as can be seen from the longitudinal section shown in figure 78.

The histological structure of the ridge has been studied in *Mytilus* (List, 1902; Tullberg, 1881), in *Anodonta* (Moynier de Villepoix, 1895; Rassbach, 1912), and in the Portuguese oyster *Crassostrea* (*Gryphaea*) *angulata* (Leenhardt,1926). Leenhardt and Moynier de Villepoix call the structure "bandelette paleale" (pallial strip) but the term subligamental ridge seems to be more descriptive.

In *C. virginica* the subligamental ridge is always well developed and easily recognizable by its shape and by its coloration, which is usually darker than that of the adjacent part of the mantle. The epithelium (fig. 78, ep.) covering the ridge presents a most striking picture. It consists of a layer of extremely tall and narrow cells arranged in fanlike groups and set on a well-developed basal elastic membrane. The length of the cells varies from 50 to 200 μ depending on the position they occupy within the layer. The cells are very thin, with granular protoplasm and an oval-shaped nucleus. At the distal portion of the ridge the boundaries of the cells become indistinct and their protoplasm darker, presumably due to the concentration there of the organic material which they secrete. The free surface of the epithelium is not attached to the ligament as was described by Moynier de Villepoix (1895).

At regular intervals the row of epithelial cells is interrupted by oval-shaped pockets which appear to be empty, with the exception of occasional amoebocytes and a few connective tissue cells. The significance of these pockets is not clear. The elastic membrane under the epithelium, thicker here than in the other parts of the mantle, includes many muscle fibers arranged parallel to the length of the ridge (m.). Large oval cells containing yellow-brownish granules (pig. c.) are abundant. The ridge is well supplied with blood through a large blood vessel (bl. v.), around which the connective tissue consists of tightly packed globular and spindle-shaped cells. Directly under the basal membrane of the ridge, however, the connective tissue of the mantle is made up of large vesicular cells.

FUNCTIONS OF THE MANTLE

Ciliary currents along the inner surfaces of the mantle form a definite pattern which may be easily observed. If one valve of the oyster is removed the corresponding mantle rolls up and exposes the gills and the inner surface of the mantle on the opposite side. In such a preparation the intact lobe of the mantle remains fully stretched and the ciliary currents can be observed by sprinkling the surface with small quantities of carmine, colloidal carbon, powdered shell material, carborundum, or other powders insoluble in sea water. It is best to use very fine particles, such as powdered mineral willemite and colloidal carbon. Willemite phosphoresces a brilliant green under ultraviolet light, which makes it possible to locate even the tiniest particles not otherwise recognizable. As a source of ultraviolet light I used a small Mineralight lamp. Hard and heavy particles of this mineral may stimulate the cilia by their weight, but this difficulty is avoided by using colloidal carbon.

As can be seen from the diagram in figure 90, drawn from life, the general direction of the currents is from the base of the mantle to its periphery, with the ciliary motion strongest in the anterodorsal sector. In the large oyster (5 inches in height) used for the drawing, this area extended along the margin of the mantle from the level of the labial palps approximately halfway down the anterior side. The upper part of the mantle was usually completely cleared 2 or 3 minutes after it was sprinkled with powder, while in the same specimen 5 to 10 minutes were required to clear the lower (ventral) part. Although the ciliary currents along the posterior side of the mantle in the area adjacent to the cloaca are also directed from the base toward the periphery, this area is swept clear by an exhalant current from the gills (fig. 90, long arrow) which is much stronger than those produced by the mantle epithelium.

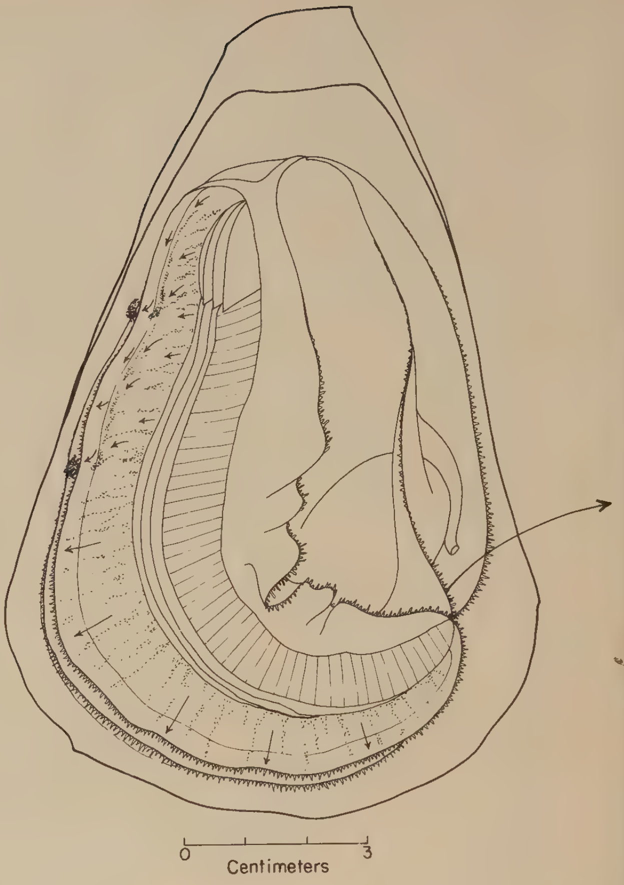

FIGURE 90.—Discharge areas of the mantle. Note the two large lumps of the discarded material at the edge of the mantle which mark the boundaries of the principal discharge area. Small arrows indicate the direction of ciliary currents. The clear path along the periphery of the mantle in the upper left side, indicated by short arrows, marks the ciliary tract located over the circumpallial artery. Long arrow at right indicates the direction of exhalant current of the cloaca. Drawn from life.

The currents along the anterodorsal part of the mantle (upper left of the figure) adjacent to the labial palps are directed at an acute angle to its free margin. There is also a well-defined tract of ciliary movement about 1.5 mm. wide parallel to the edge of the mantle. Upon reaching the level of the lower corners of the labial palps this current turns sharply about 90 degrees across the mantle edge. This point marks the upper limit of the area throughout which the material settled on the mantle is discarded; in the oysters which received powdered suspensions large lumps of particles entangled in mucus are usually seen here. A similar accumulation of material ready to be discarded marks the anterior boundary of the discharge area which may vary in dimension and location in different oysters.

Along the ventral portion of the oyster body the ciliary currents sweep across the mantle at right angles to its edge and material is discarded along the border. In this respect my observations differ from those of Nelson (1938, p. 24), who thinks that the current in this area is directed along the free margin toward the mouth. Such a current was not present in the large oysters (*C. virginica*) I used in my experiments.

The existence of a special discharge area located in the zone below the labial palps is biologically significant. The so-called pseudofeces, or masses of discarded food particles and of detritus entangled in mucus, which accumulate in this area either slide over the free edge of the mantle and drop off or are forcibly ejected by the snapping of the valves. There is no doubt that the presence of pseudofeces near the edge of the mantle stimulates the strong ejection reaction which constitutes one typical pattern of shell movement in the oyster (see p. 169).

FORMATION AND CALCIFICATION OF SHELL

The principal function of the mantle is the formation of the shell and its calcification. The great structural complexity and intricate pattern of pigmentation found in some species are produced by the mantle. The regulatory mechanisms responsible for this process are not known because the morphogenesis of molluscan shells has never been studied experimentally. From observations on shell growth in some gastropods and lamellibranchs it is clear that the shape of the shell as well as the pattern of pigmentation result from the position assumed by the edge of the mantle during periods of shell secretion and from the rate of deposition of calcium salts and pigments.

It can be easily observed in oysters, scallops, and other bivalves in which the edges of the mantle are not fused together that during periods of growth the mantle extends a considerable distance beyond the border of the shell. In some species it even stretches far out and folds back over the outer surface of the valve. In this way, for instance, the mangrove oysters produce hooks or similar structures by which they attach themselves to branches of trees (fig. 5).

The differential rate of growth along the periphery of the shell as well as the formation of spines, nodes, ridges, and similar sculptural elements are both caused by changes in the rate of deposition of shell material. Two distinct phases may be distinguished in the shell-forming process: (1) the movements of the mantle which stretches and folds itself in order to provide a matrix or mold upon which the shell is formed, and (2) the secretion and deposition of the shell material itself. It is probable that the circumpallial nerve plays a role in the first phase of the process by controlling the muscular activity of the mantle. Our present knowledge of the physiology and biochemistry of shell secretion is inadequate to propose an explanation of the morphogenetic processes involved in shell formation. These processes are not haphazard but follow a definite and predetermined course. This is self-evident from the fact that the final shape of the shell has definite mathematical characteristics (see p. 24) which can be attained only by orderly and regulated deposition of organic framework and mineral salts.

The first step in the formation of the oyster shell is the secretion of conchiolin from the periostracal gland. This process can be easily observed by cutting off a small section of the edge of the upper valve and exposing the intact valve and the underlying mantle of the opposite side. Under a low-power binocular microscope one can see a clear, viscous, and sometimes stringy substance oozing out of the periostracal groove. While secretion is taking place the edge of the mantle appears to be very active, expanding and retracting as successive layers of conchiolin are laid down. Figure 91 shows the position of the mantle at the time of its retraction.

The newly deposited shell (n.sh.) extends outward along the plane of the valve; the edge of the mantle (mn.e.) rolls upward; its outer lobe (o.mn.l.) is parallel to the plane of the valve, while the middle and inner lobe (m.l.) face the observer. The tentacles of the inner lobe extend down; those of the middle lobe are slightly contracted. The outer lobe underlies the sheet of

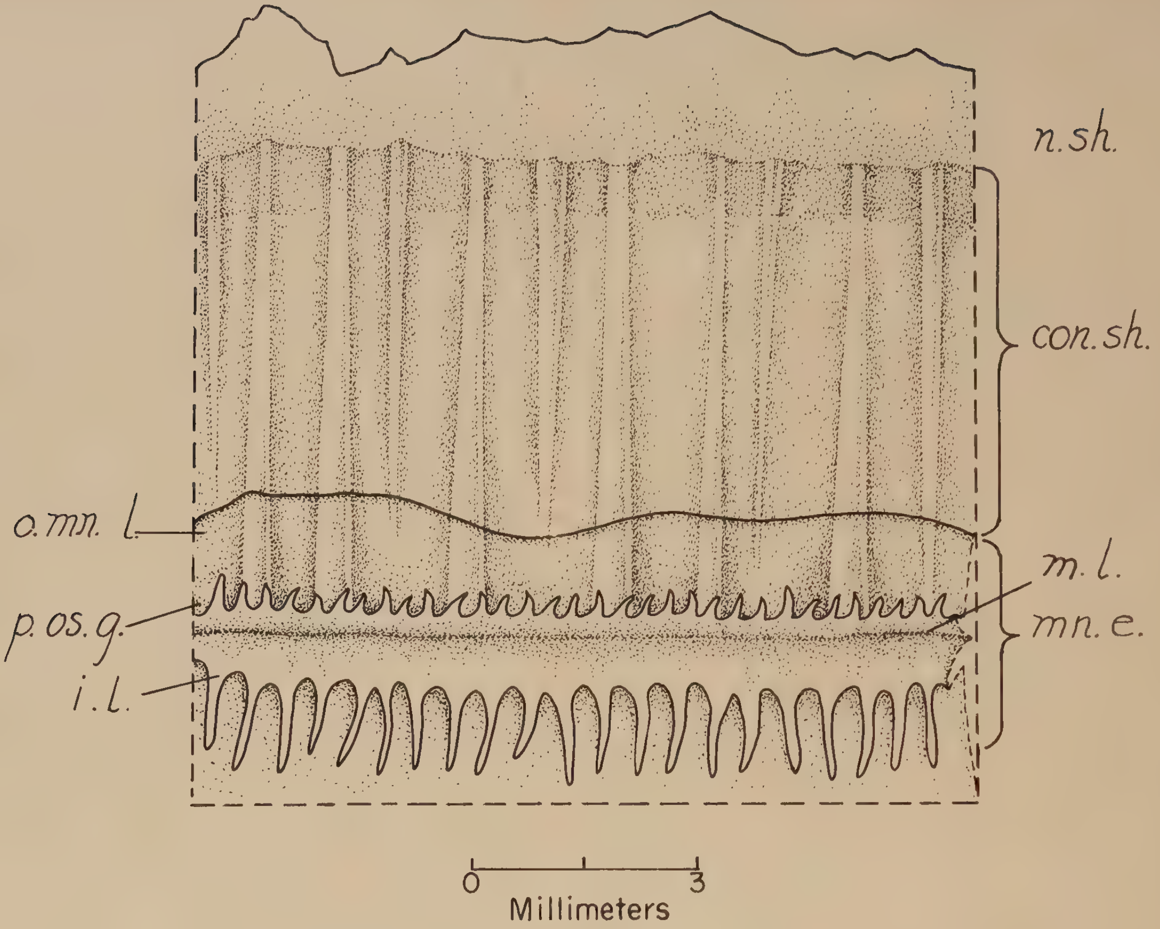

FIGURE 91.—Small area of the mantle edge with the adjacent part of the newly secreted shell viewed from above. The mantle was exposed by cutting off a piece of the opposite valve, and the oyster was placed in sea water under a binocular microscope. con.sh.—conchiolin sheet; mn.e.—edge of the mantle; i.l.—inner lobe; n.sh.—new shell; o.mn.l.—outer lobe of the mantle, contracted; p.os.g.—periostracal groove. Drawn from life. The position of structures at the edge of the mantle in relation to one another: the new shell area (n.sh.) marked on three sides by a broken line is in the plane of the drawing, next to it is the outer lobe (o.mn.l.), then the conchiolin sheet (con.sh.), the middle lobe (m.l.), and the inner lobe (i.l.) is at the top, nearest to the observer.

viscous conchiolin (conch.sh.) which oozes out from the periostracal groove (p.os.g.) between the outer and middle lobes. The distal edge of the conchiolin sheet (end of stippled area) indicates the previous maximal extension of the outer lobe before the withdrawal of the mantle edge. The entire group rests on the newly formed and already solidified shell (n.sh.).

During the secretion of conchiolin the edge of the mantle frequently extends out and then withdraws to the position recorded in the drawing. At the time of expansion the outer lobe temporarily supports the semiliquid conchiolin and by moving in and out spreads it over the shell. Because of this action the proximal part of the newly formed valve receives a larger amount of conchiolin and becomes thicker than the distal portion. When secretion is interrupted, the conchiolin layers become incorporated into the shell substance and the conchiolin sheet as shown in figure 91 is no longer visible.

The rate of secretion of the new shell varies at different parts of the mantle edge. Quantitative data are lacking, but observations made during the periods of more rapid growth in *C. virginica* (May to June and October to November in New England waters) show that the area of newly formed shell is always largest at the ventral side of the valves near the principal axis of growth (fig. 92).

The organic matrix of the shell can be produced by the pallial epithelium at any place along the

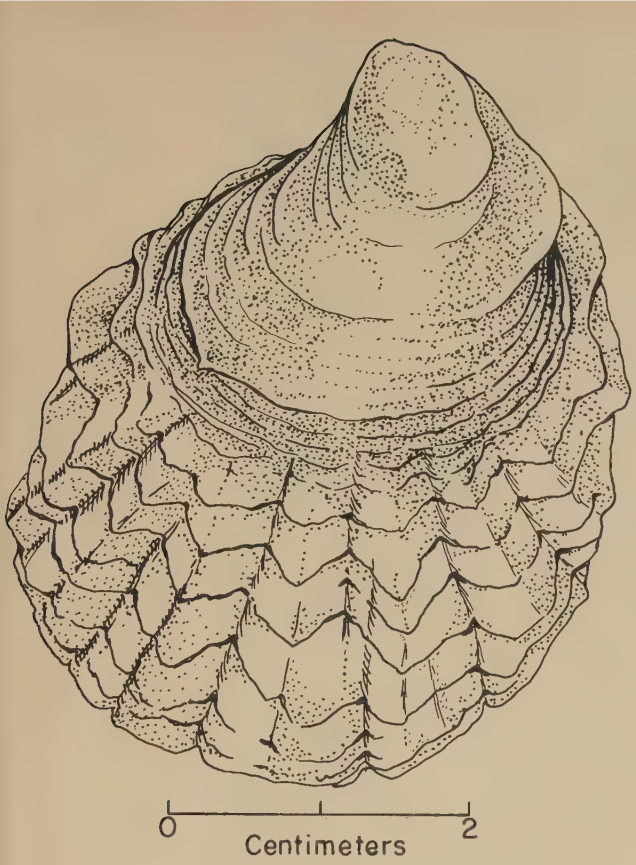

FIGURE 92.—New shell growth formed during 1 year along the periphery of the valve of an adult oyster from Long Island Sound planted in the Oyster River, Chatham, Mass. The newly formed shell is recognizable by zigzag lines of the material; its width is greatest along the ventral edge.

entire outer surface of the mantle and is not restricted to the periostracal groove. Such secretion, first observed in pearl oysters (Bøggild, 1930), can be experimentally demonstrated in *C. virginica*. Oysters with one valve removed and the edges of the mantle cut off above the periostracal groove secreted a new conchiolin layer over the entire surface of the exposed mantle within 5 days. Although the operated specimens remained alive in the laboratory tanks at Woods Hole over 3 weeks this conchiolin membrane remained uncalcified. In another experiment three adult oysters were removed from their shells and kept alive in sea water for 3 weeks. They formed rather thick coats of periostracum which was very lightly calcified. The repair of holes made in oyster shells by boring snails and sponges also shows that conchiolin is secreted by the entire surface of the mantle. The damaged area is rapidly covered by a layer of organic material which later becomes calcified.

Soon after being secreted, the conchiolin becomes calcified. Progressive stages of this process can be observed on the growing edge of the shell, or by inserting pieces of plastic or small glass cover slips between the edge of the mantle and the valve and removing them at regular intervals for inspection. The earliest stage of calcification is recognized by the appearance of minute granules of calcium salts, which become visible in polarized light as brightly sparkling dots (fig. 93). At this early stage the distribution of the granules (calcospherites) does not show any definite pattern or arrangement. In a living oyster they can be found entangled in strands of mucus left on the conchiolin sheet by the back and forth movements of the mantle edge. Within the next 24 to 48 hours typical hexagonal crystals of calcite can be seen (fig. 94, black crosses). They gradually increase in size and present a picture of great brilliance and beauty in polarized light (fig. 95).

Distribution of calcospherites at the stage of their transformation into small calcite crystals on the surface of the newly secreted shell (fig. 96) does not show any distinct orientation in relation to the growth axis of the shell. Some of the calcospherites are scattered over the entire field of vision, while others are packed tightly between the larger crystals (see large group of crystals at the lower part of figure 96). Within the next 48 hours the calcite crystals increase in size (fig. 97). In the final stage of shell formation the calcite crystals become arranged in a distinct pattern to form the prismatic layer in which each unit is a prism oriented with its long axis at about a 90° angle to the edge of the shell (fig. 98). The form of the individual prisms varies greatly, some of them are even wedge-shaped and slightly curved. This can be observed after boiling a piece of shell in a strong sodium hydroxide solution to separate the prisms (Schmidt, 1931).

Each calcite prism is surrounded by a capsule of conchiolin. By dissolving the mineral in weak hydrochloric acid it is possible to obtain intact the organic meshwork of the conchiolin layer. The walls of each capsule, as can be seen in figure 99, are very thin and slightly iridescent. Since in the earliest stages of shell formation the conchiolin sheet appears to be amorphous under the light microscope, it is reasonable to assume that the organic capsules of the calcite prisms are formed

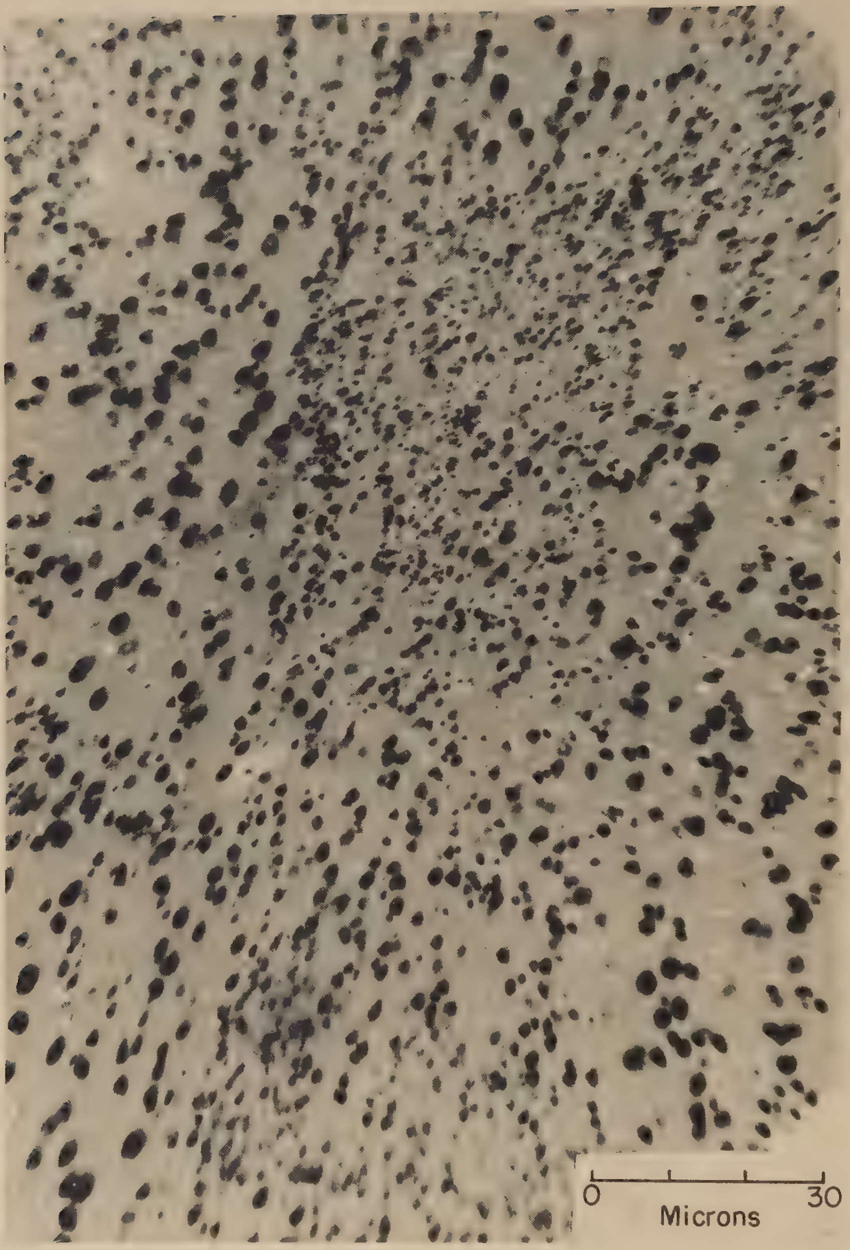

FIGURE 93.—Small granules (calcospherites) in conchiolin shortly after its secretion by the mantle. Black and white enlargement of a Kodachrome photograph taken with polarized light.

by later deposition of conchiolin, the secretion of which continues during calcification. The details of this prosess have not yet been described.

THEORIES OF CALCIFICATION

Studies of shell calcification fall into two major categories. One type of work places the emphasis on the identification of calcium-secreting cells or organs; the other approaches the problem from the biochemical point of view. It has been generally accepted that calcium carbonate, separated from blood, is secreted as colloidal gel by certain cells at the edge of the mantle and that crystallization takes place outside the cells (Crofts, 1929; Dakin, 1912; Kuyper, 1938) between the conchiolin sheet and the mantle. Separation of calcium is not, however, confined to the surface cells of the mantle. The calcium-secreting cells may be subepithelial, as in *Patella* (Davis and Fleure, 1903). In the calcification of the epi-

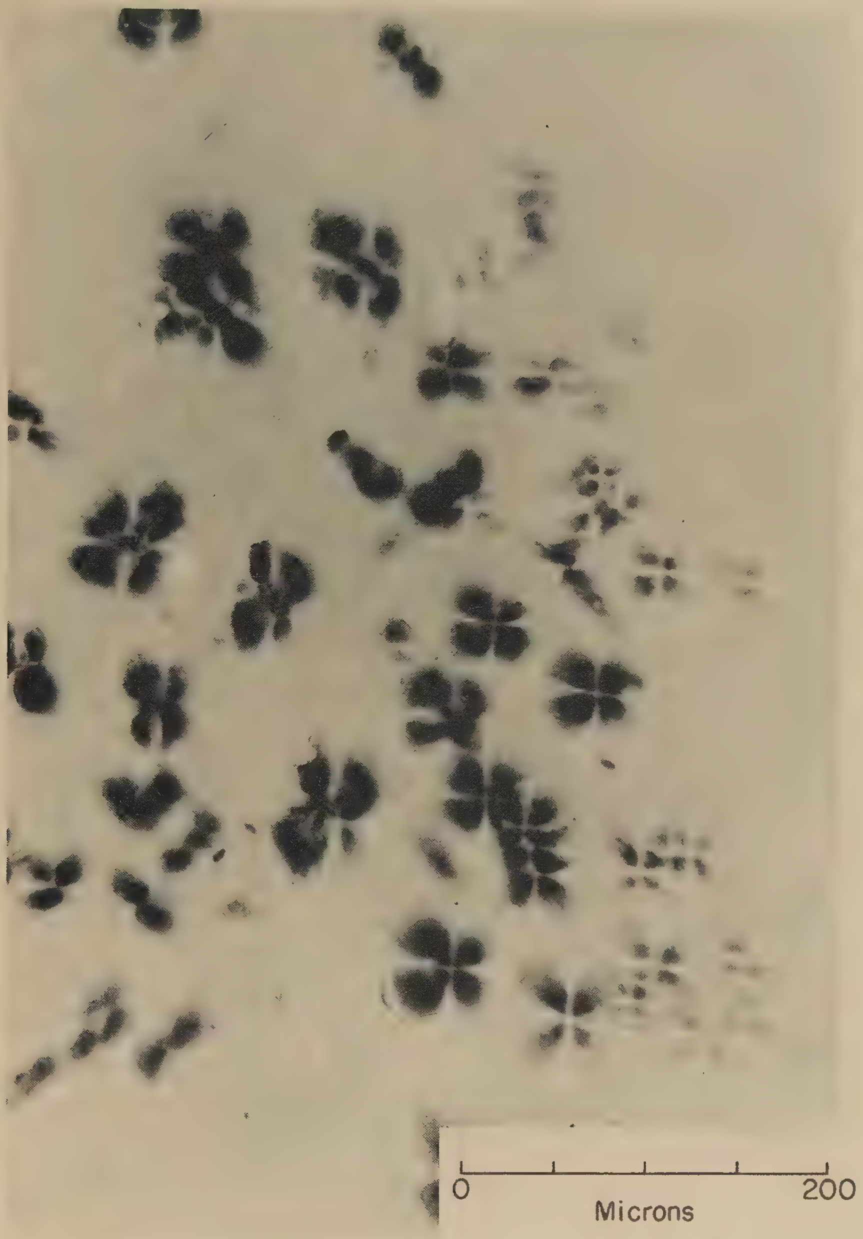

Figure 94.—Calcite crystals of new oyster shell about 24 to 36 hours after its formation. Black and white enlargement of a Kodachrome photograph taken with polarized light.

phragm of *Helix pomatia* (Prenant, 1924, 1928), the calcium is liberated by the leucocytes in the connective tissue of the mantle. In the case of pearl formation, Boutan (1923) has shown that calcareous deposits are formed by amoeboid cells which crawl through the mantle epithelium, while the latter secretes the concentric layers of the organic matrix (conchiolin).

De Waele (1929) approached the calcification problem from the physiochemical point of view. Working with *Anodonta cygnea* he has shown that the extrapallial fluid between the mantle and the shell is chemically identical with blood. Exposure of this fluid to air causes the formation of a precipitate, which consists of a suspension of calcium spherules in protein solution. He therefore assumed the existence in the pallial fluid of a hypothetical compound consisting of protein, carbon dioxide, and calcium carbonate. The escape of carbon dioxide would then cause the

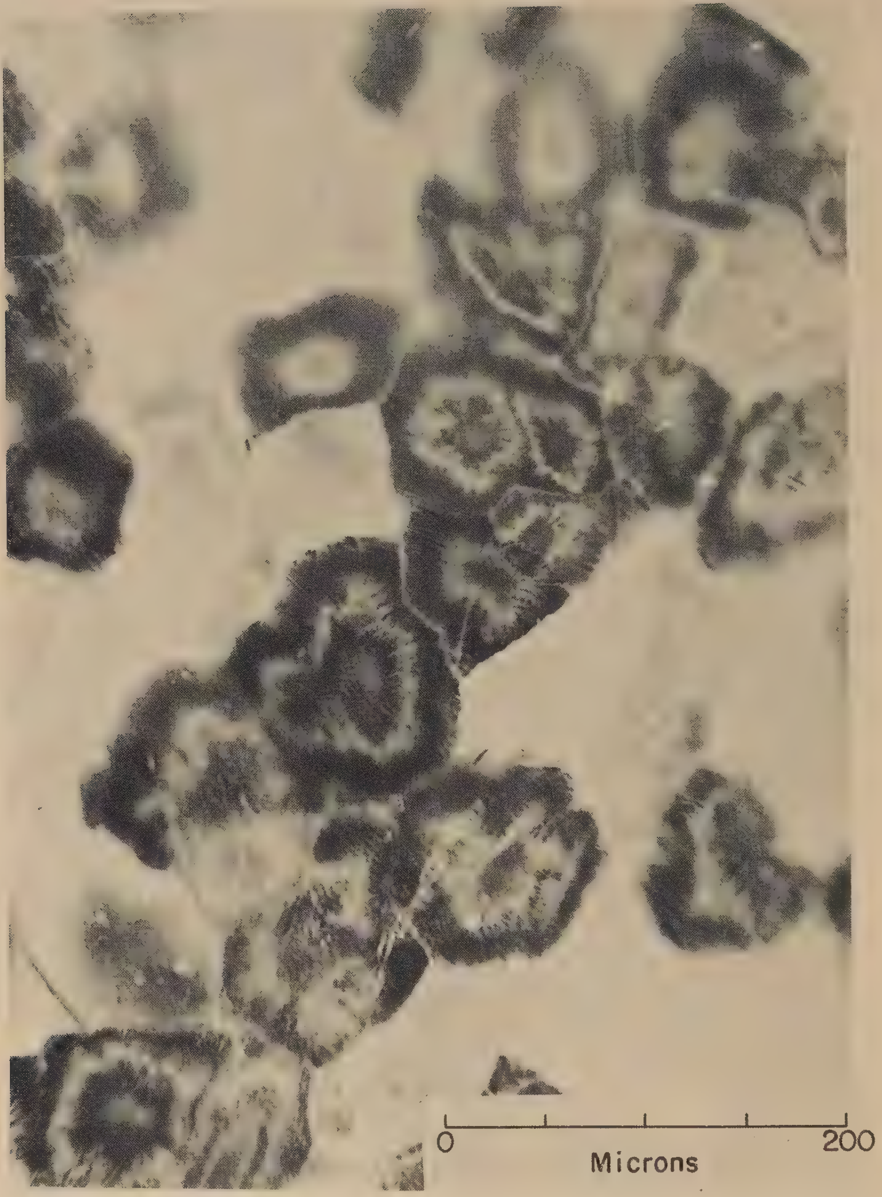

FIGURE 95.—Early stage of the formation of prismatic layer. Photographed with polarized light.

precipitation of calcium carbonate. Dotterweich and Elssner (1935) found, however, that calcium carbonate crystals are formed in the extrapallial fluid of *Anodonta* only in an atmosphere containing less than 1.5 percent carbon dioxide. In *Helix*, regeneration of the shell will take place in an atmosphere containing up to 15 percent of carbon dioxide, according to Manigault (1933). Although the latter accepted De Waele's theory, his own results seem to prove its inadequacy; and Robertson (1941) remarks that De Waele's hypothetical protein compound is without a real chemical basis. Furthermore there are other discrepancies in De Waele's results which invalidate his theory. The calcospherites and the protein precipitated from blood and from extrapallial fluid contained 50 percent organic matter, whereas the new shell contained only 4 percent of it. To reconcile these facts it would be necessary to assume that a great proportion of the organic matter in the new shell must be reabsorbed. The entire process as outlined by De Waele appears to be highly improbable.

Steinhardt (1946) assumed that calcification of the oyster shell is associated with the formation of citrate, probably the tricalcium-citrate

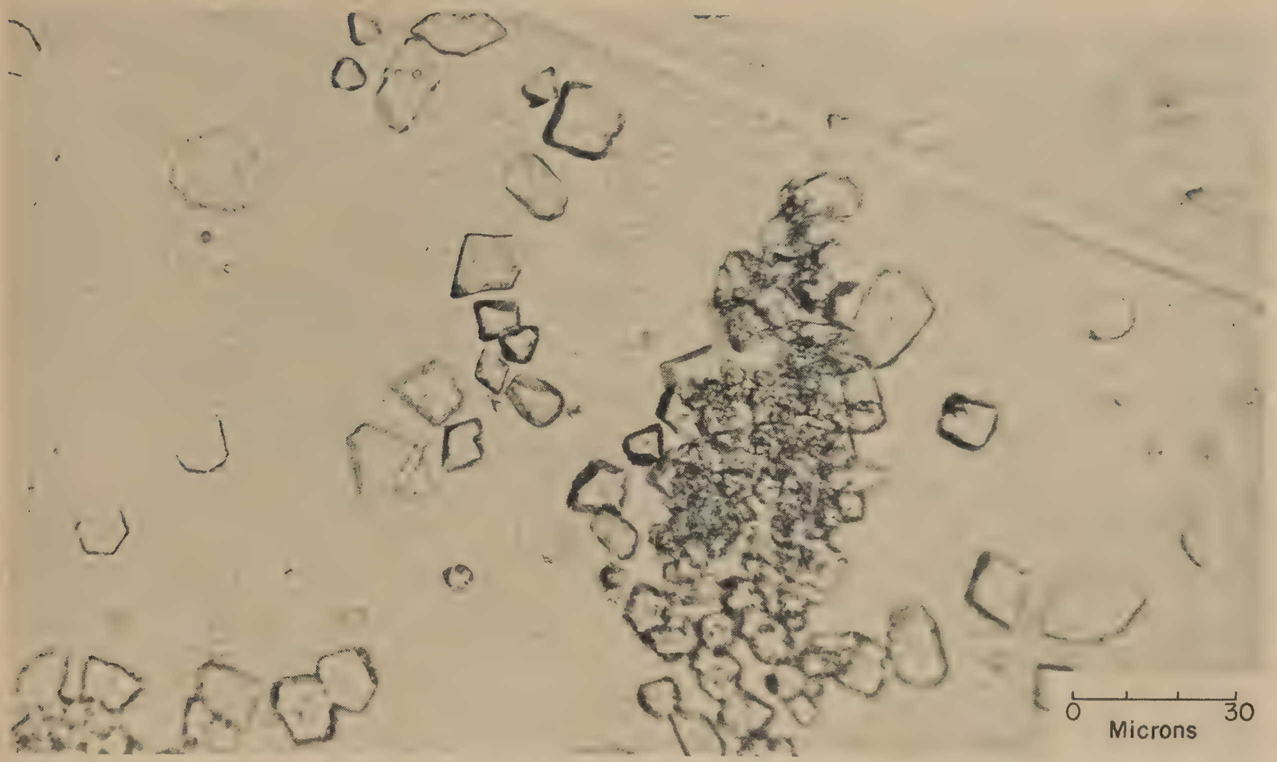

Figure 96.—Photomicrograph of a piece of new shell of *Crassostrea virginica* taken 28 hours after the beginning of calcification. Small calcite crystals are randomly distributed, and calcospherites scattered over the entire field of view are in places densely packed between the larger crystals.

$(C_6H_5O_7)_2Ca_3+4H_2O$. The observation that citric acid is formed in connection with carbohydrate metabolism, and that citrate is qualitatively precipitated from a solution which also contains phosphate and calcium ions in a suitable concentration (Kuyper, 1938, 1945a, 1945b), forms the basis of his conclusion. The citrate in the precipitate is found not as calcium citrate but in a somewhat more complex form in which calcium is combined with both phosphoric and citric acids. This is verified by the results of the analyses shown in table 11, in which the oyster shell was presumably *O. edulis*. It is rather difficult to arrive at a definite conclusion regarding the role of citric acid in the calcification of oyster shells, but Steinhardt's observations establish the presence of calcium phosphate in the oyster shell, which was supposed to consist primarily of carbonates; and an abundance of calcium phosphate in the mantle was demonstrated by Biedermann (1914).

During recent years (Bevelander, 1952; Bevelander and Benzer, 1948; Bevelander and Martin, 1949; Hirata, 1953; Jodrey, 1953) considerable advances in the study of the processes of calcification have been made. It had been generally assumed that the small granules appearing on the surface of the conchiolin consisted of calcium carbonate, but Bevelander and Benzer found that they are made of calcium phosphate. It is not at all clear how the calcium phosphate of the granules is converted into calcium carbonate, which is the final product of calcification in the oyster shell. It is doubtful that the conversion is accomplished by direct reaction between the calcium phosphate and the carbonate, because such a process would require very high concentrations of carbonate. The explanation proposed by Bevelander and Benzer implies that calcium phosphate may be dissolved by the action of organic ions which in some manner bind calcium. Phosphatase may

Table 11.—*Analyses to calcified materials according to Steinhardt*

[All figures are in percentages]

Material	Citric acid	Phosphorus	Calcium
Concretions from crayfish stomach	1.56	9.0	25.6
Chicken egg shell	0.15	0.154	33.3
White coral	0.013–0.024	0.007	35.2
Oyster shell	0.017	0.019	32.8

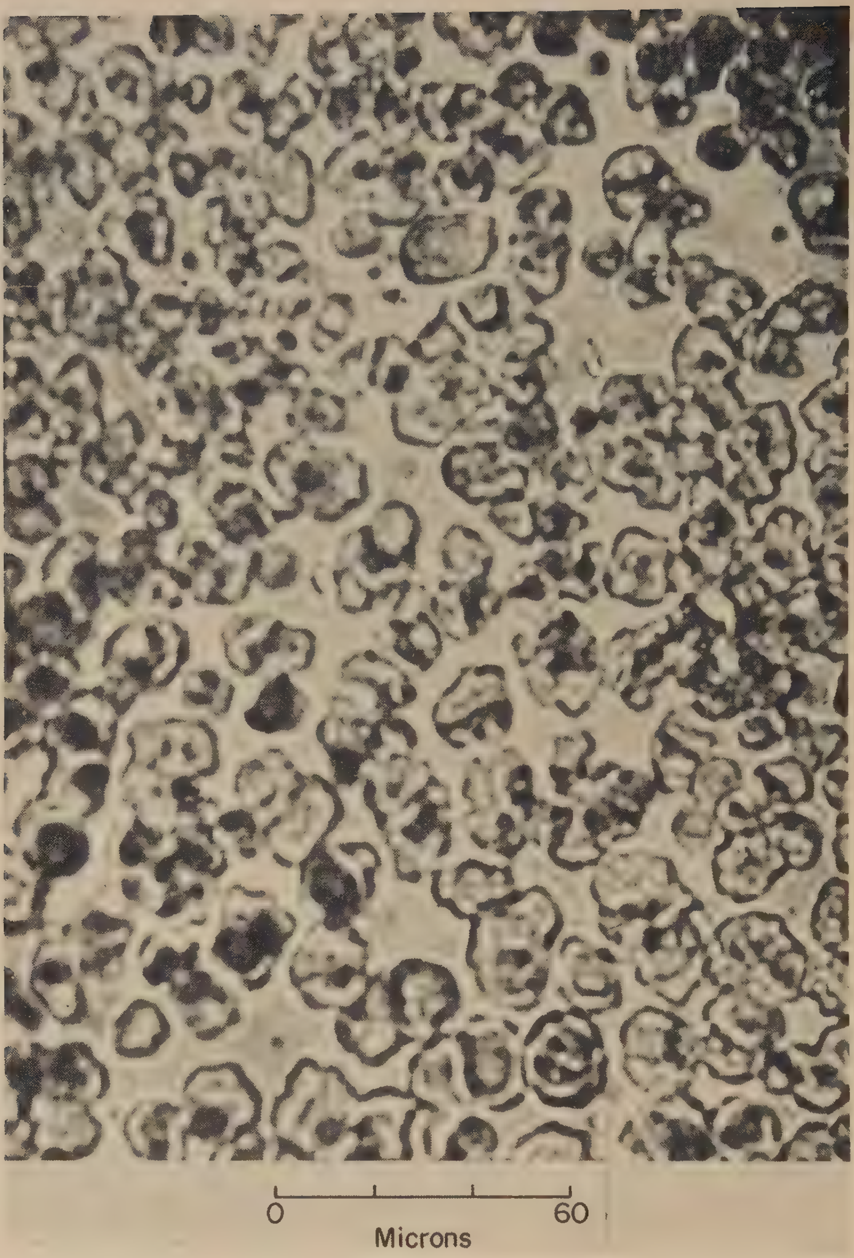

FIGURE 97.—Calcite crystals deposited on a piece of conchiolin. Photomicrograph taken 38 hours after the secretion of conchiolin has started.

contribute to this process by transferring phosphate to some substrate and removing the phosphate ions. This tentative explanation suggests a number of biochemical studies that should be made to obtain a better understanding of the process of calcification.

An important factor in the process of shell calcification is the enzyme phosphatase, which is generally present in the ossifying cartilages of young animals and in other tissues and organs in which calcium is deposited. The action of the enzyme consists of hydrolysis of hexosemonophosphoric ester and glycerophosphoric ester and consequent liberation of inorganic phosphate. The role of phosphatase in the shell formation of mollusks was established by Manigault (1939), who found a direct correlation between phosphatase activity in the digestive diverticula, mantle, and blood and precipitation of calcium in the shell. He concluded that phosphatase is

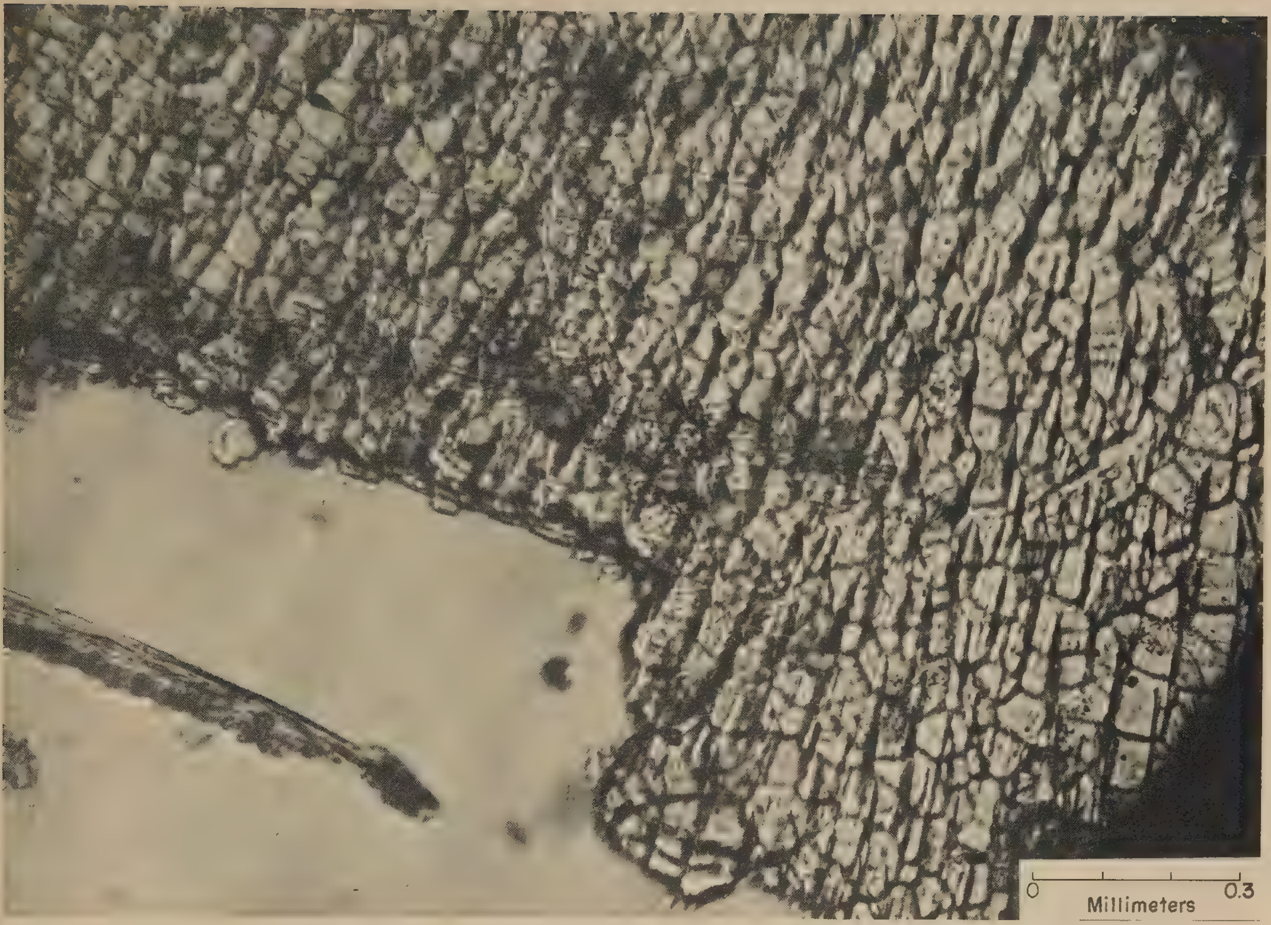

FIGURE 98.—Prismatic layer of the new growth of shell at the edge of the mantle. Four to five days old. Photomicrograph of an unstained whole mount.

probably a transfer agent involved in the mobilization of calcium. The localization of this enzyme along the border of the mantle and in the surface epithelium of the oyster, shown by the Gomori technique (fig. 80), confirms the opinion of Manigault and of Bevelander that the phosphatase plays an active role in the calcification of oyster shells.

During the last decade considerable advance was made in studies of the metabolic aspects of shell formation. Hammen and Wilbur (1959) paid particular attention to carbon dioxide conversion to shell carbonate and to the secretion of conchiolin matrix in which the calcium carbonate crystals are deposited by the oyster (*C. virginica*). The work of Jodrey and Wilbur (1955) on high activity of the enzyme oxalocetic decarbohylase in the mantle tissue of this species suggested that the deposition of carbonate may be related to decarboxylation reactions of the mantle. Experimental work conducted by Hammen and Wilbur at the Duke University Marine Laboratory at Beaufort, N.C., corroborated this hypothesis. Living oysters and isolated shells were placed for 12 hours in sea water containing 240 microcuries of $NaHC^{14}O_3$ per liter. The radioactivity of the shell surface was determined near the posterior margin of the right valve and corresponding correction was made for self absorption on the surface. By incubating pieces of oyster tissues in $NaHC^{14}O_3$ it was found that C^{14} is incorporated into organic acids of the mantle. More than 90 percent of the radioactivity occurs in succinic and smaller amounts in fumaric and malic acids. The initial step in the process is the fixation of carbon dioxide by propionic acid resulting in the formation of succinic acid. Both acids were found in relatively high concentrations in the shell forming tissues of the oyster. The fact that in these experiments labeled amino acids were found in the radioactive

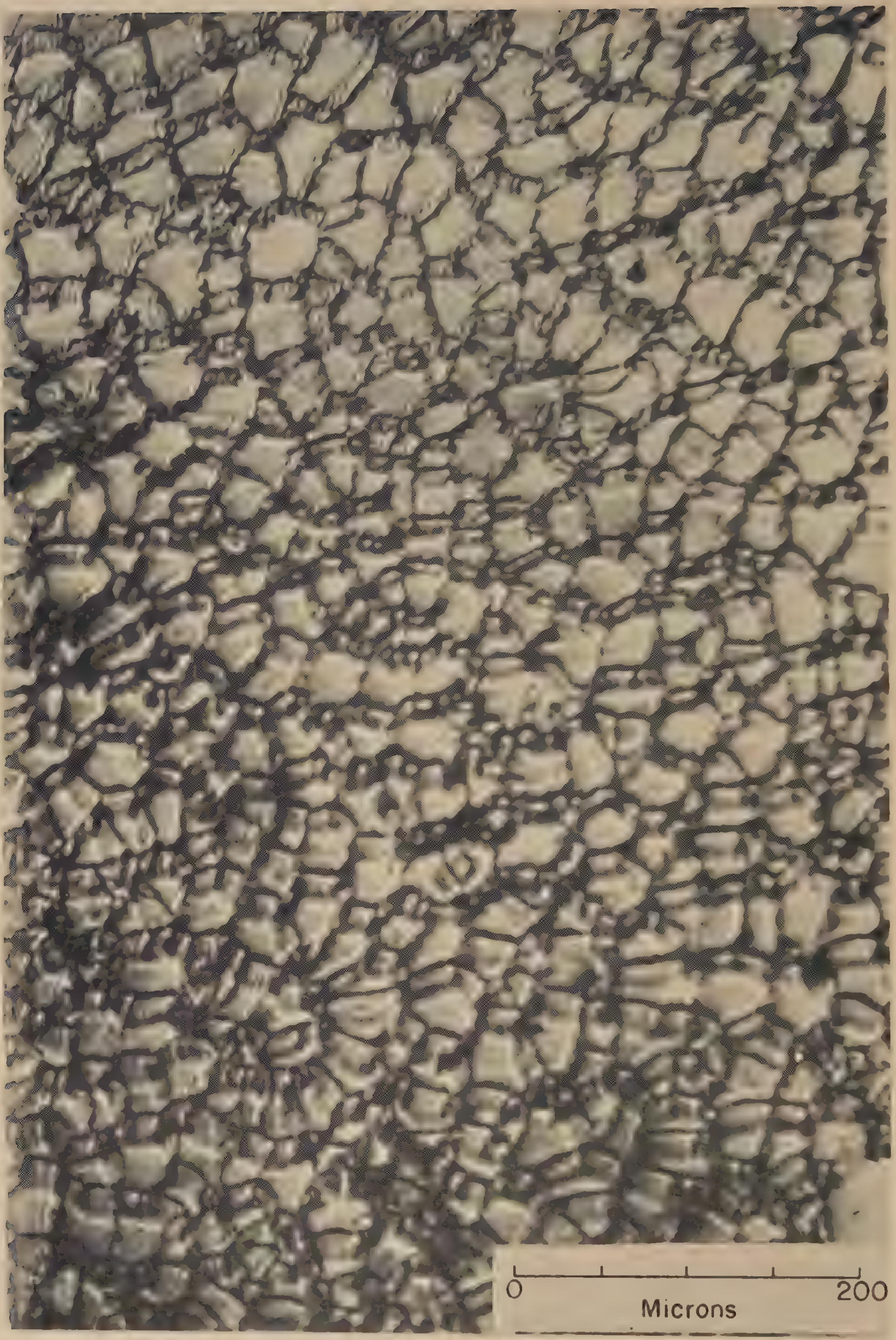

FIGURE 99.—Photomicrograph of organic meshwork of prismatic layer of shell after decalcification in weak hydrochloric acid. Note that the outlines of the capsules retain the shape of the mineral prisms.

conchiolin of the shell indicate that carbon dioxide fixation also contributes to the syntheses of the organic matrix of the shell.

Calcium enters the mantle directly from sea water, as was demonstrated by Jodrey (1953) using mantle-shell preparation and radioactive Ca^{45}, and can be taken up through other parts of the mollusk and transported to the mantle. The enzyme carbonic anhydrase which is present in various mollusks may be expected to accelerate deposition of calcium carbonate, and the rate of deposition is retarded by carbonic anhydrase inhibitors.

Complex metabolic cycles involved in shell formation have been reviewed by Wilbur (1960), and probable relations of carbon dioxide to shell conchiolin and carbonate deposition are shown by him in a summary diagram (fig. C, p. 25 of Wilbur's paper).

CYTOLOGICAL IDENTIFICATION OF CALCIUM

Several methods for the identification and localization of calcium salts in the oyster tissues are available, but none are completely reliable. Gomori (1939) suggests that soluble calcium could be demonstrated by treating the frozen sections with ammonium oxalate, the insoluble octahedral crystals of calcium oxalate being easily recognized. The use of a fixative consisting of formalin and ammonium oxalate was also proposed (Rahl, quoted from Gomori). Both methods tried in my laboratory on sections of oyster mantle gave unsatisfactory results. The difficulty is the dislodging of calcium-bearing granules and mucus during sectioning, since the granules are easily carried out by the knife's edge from their original location inside the cells to the outside of the epithelium. This difficulty can be avoided to a certain extent by double embedding the tissue in colloidin-paraffin.

Indirect methods of Ca^{++} identification are based on the use of heavy metals (silver, cobalt, copper, and iron). Because almost all insoluble calcium compounds in the tissues are either phosphate or carbonate, any procedure which would demonstrate the presence of these anions may be considered specific for calcium. When the sections are immersed in a solution of one of the heavy metals the corresponding metallic salt is formed at the sites of phosphate or carbonate. The reduction may be effected by exposing to light if silver nitrate is used, or by immersing in appropriate reducing reagents (ammonium sulfide, acidified potassium ferricyanide). Identification by staining of calcium is based on the formation of insoluble lacs with several hydroxyanthraquinine dyes (alizarin sulfonic acid, purpurin, anthrapurpurin). Calcium deposited in the process of shell formation may, however, contain substances which interfere with the lac-forming reaction of alizarin. Also, the dye frequently fails to stain old deposits and its color is affected by the presence of iron. Although these complications limit the usefulness of alizarin as a reagent for the determination of calcium, I found that a 1 percent water solution of alizarin S (sodium alizarin sulphonate) is probably the best histochemical reagent for identification of calcium in the oyster mantle. It readily reacts with new deposits of calcium carbonate or calcium phosphate and forms compounds resistant to both acids and alkalies.

To study the cytology of calcium secretion, the deposition of conchiolin and its calcification was stimulated by cutting off small pieces of shell along the posterior margin of the oyster. Laboratory experience shows that such injury made during the warm season is rapidly repaired. Small pieces of the mantle border with the adhering and partly calcified conchiolin were excised and 3 days later preserved in neutral formalin or absolute ethyl alcohol. Sectioned tissues were stained with alizarin S and other reagents for demonstration of calcium. The preparations showed a large number of alizarin stained globules or granules, about 1.5 μ or less in diameter adhering to the surface of the mantle. Identical granules were found inside the goblet cells of the epithelial layer along both sides of the mantle (fig. 100).

The results of the staining and other histo-

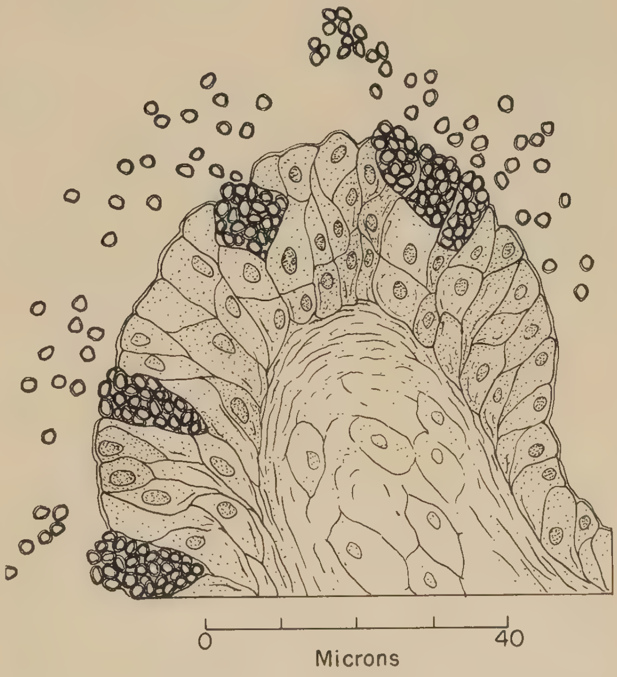

FIGURE 100.—Calcium containing granules discharged by the epithelial cells at the edge of the mantle of *C. virginica*. Neutral formalin 3 percent, alizarin.

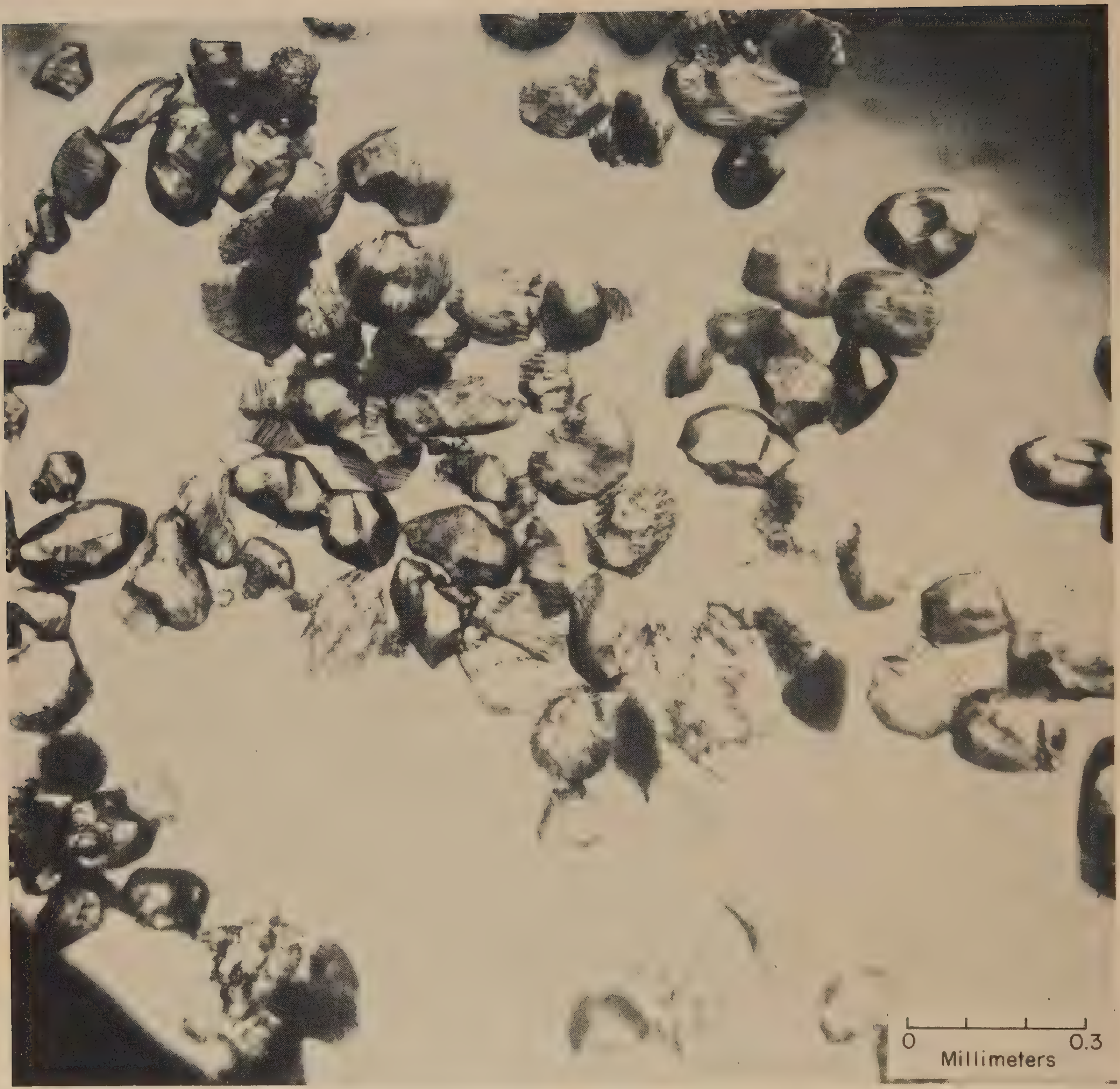

Figure 101.—Photograph of crystals of a mixture of calcite and gypsum formed in the mantle cavity of *C. virginica.*

chemical reactions show that the secretion of calcium is not confined to special sites but takes place over the entire edge and outer surface of the mantle. The intensive coloration of the granules by alizarin suggests that they contain a considerable amount of calcium, probably bound in organic compounds of the globules. Amoebocytes present in the material secreted by the mantle also may be involved in the mobilization of calcium during the formation or repair of shells.

Sometimes the mineral crystals formed by the mantle are not incorporated in the conchiolin but accumulate in the pallial cavity and are eventually ejected. On several occasions fairly large quantities of a white powdered material were found in front of the discharge areas of oysters which were kept in glass trays in running sea water in the laboratory. The material consisted of crystals (fig. 101) which, according to the X-ray analysis kindly performed by Marie Lindberg of the Geochemistry and Petrology Branch of the Geological Survey of the U.S. Department of the Interior, were found to consist of a mixture of calcite and gypsum (hydrous calcium sulfate), with the latter present only as a minor constituent. The oysters appeared to be normal in every respect and showed good growth of shells. The presence of gypsum is of interest since it is not a normal constituent of oyster shell. What particular disturbance in the calcium metabolism produced its formation is unknown.

SOURCES OF CALCIUM

It has been suggested (Pelseneer, 1920; Galtsoff, 1938) that lamellibranchs may remove calcium directly from sea water. Pelseneer (1920) cites an example of a young *Anodonta cygnea* which in 2 months removed all the calcium from 5 l. of water in which it was kept. Definite proof of the direct absorption of calcium by the oyster mantle is given by the experiments with *C. virginica* (Jodrey, 1953) in which radioactive Ca^{45} was used. Calcium turnover was also studied by Hirata (1953) in mantle-shell preparations made by cutting off the adductor muscle and the visceral organs, and leaving the intact mantles spread over their respective valves. The mantle remained alive for several days and deposited the shell material, although at a lower rate than does the intact oyster. Jodrey placed a mantle preparation in 500 ml. of aerated sea water with a Ca^{45} activity of 5.8 microcuries. At least part of the calcium of the newly formed shell substance came directly from the sea water, and the deposition of calcite took place in tissue isolated from the circulatory and digestive systems. The experiments also demonstrated that the greater portion of calcium in the mantle appears to be inert. Only 2.5 percent of the total calcium content was renewed every 24 minutes, the turnover being 0.6 mg. of calcium per minute per gram of mantle. In addition to entering the mantle directly calcium can be taken up by other organs of the oyster and transported to the mantle (Wilbur, 1960).

MINERALOGY OF CALCIUM CARBONATE IN MOLLUSCAN SHELLS

Calcium carbonate is known to occur in 12 mineral forms (Prenant, 1924), but only three of these have been found in animals. In the shells of mollusks, calcium carbonate usually occurs as calcite and aragonite. There are many species in which both minerals occur together although in different parts of the shell. Prenant (1928), who contributed much to the study of calcification, found that besides calcite and aragonite the animal tissue may contain small spheres (sphaerolithes) or tiny needles of the mineral called "vaterite", after the mineralogist Vater who discovered it. Vaterite was reported to be present in the connective tissue of certain gastropod mollusks, cestodes, and trematodes, and in the fat tissue of insects (Diptera). Its presence in the tissues of the oyster has not been reported.

The various forms of calcium carbonate secreted by animal tissue can be identified by their crystallographic properties, birefringence, density, and chemical reaction. Some of these distinctive characteristics are summarized in table 12, taken from Prenant (1924).

Impurities always present in material secreted by living forms can sometimes make the mineralogical identification of calcium carbonate doubtful. Calcite and aragonite can be distinguished by means of the polarizing microscope. Calcite crystals examined under crossed nicols give a brilliant picture of various colors, and a distinct black cross appears when the optical axis is aligned parallel to the axis of the microscope (fig. 94.) In the case of aragonite, hyperbolic arched lines appear instead of the black crosses. Exact identification of minerals can of course be made by X-rays, but this method is rarely available to the biologist.

Among various chemical identification methods the Meigen color reaction can be most easily employed (Bøggild, 1930, p. 238). In a weak solution of cobalt nitrate aragonite becomes violet, the intensity of coloration increasing as the solution is warmed. Calcite, however, remains pale blue even in a heated solution.

The conditions under which a mollusk secretes calcium carbonate in a specific mineralogical form are not at present understood. It is reasonable to presume that the organic matrix of the shell is somehow involved in this process. Roche, Ranson, and Eysseric-Lafon (1951) found that in the shells of mollusks consisting both of calcite and aragonite the conchiolin associated with the calcite of the prismatic layer had higher concentrations of glycine and tyrosine than were present in the nacre of the same shell consisting of aragonite (see ch. II, p. 41). The causal relationship between the mineralogical forms of carbonate and amino acids of its conchiolin has not been demonstrated.

A hypothesis that carbonic anhydrase, an enzyme present in the tissues of the mantle, plays

TABLE 12.—*Distinctive properties of principal mineral forms of calcium carbonate found in invertebrates* [1]

Name	Chemical composition	Optical System	Birefringence	Index of refraction	Density	Meigen reaction
Calcite	$CaCO_3$	Rhomboedric, uniaxial	Strong (0.172)	1.658–1.486	2.714	Negative.
Aragonite	$CaCO_3$	Monoclinic, biaxial	Slightly weaker, (0.156)	1.686–1.530	2.95	Positive.
Vaterite	$CaCO_3$	Sphaerolithes, optically negative	Weak	About 1.55	2.5–2.65	Do.
Amorphous	$CaCO_3$	Isotropic		Near 1.5	2.25–2.45	
Hydrated carbonate	$CaCO_3\ 6H_2O$	Prisms or Monoclinic tablets	Near 0.085	About 1.5	1.777	

[1] From the data published by Prenant, 1927.

an important role in the formation of calcium deposits in molluscan shells has been advanced by Stolkowski (1951). According to this theory the enzyme exerts its effect by orienting the calcium carbonate molecules in the aragonite crystal lattice. The action of carbonic anhydrase in this admittedly very complex process is not, however, satisfactorily explained and should be more thoroughly investigated before its role in the formation of aragonite or calcite in mollusk shells is definitely established. In its present state the hypothesis fails to explain the existence of shells in which both aragonite and calcite are present. Recently Stenzel (1963) reported that in the shells of *C. virginica* aragonite covers the areas of attachment of the adductor muscle, the imprint of Quenstedt's muscle, and is found in the ligament.

Another explanation of the formation of the less stable aragonite instead of calcite suggests that strontium and magnesium carbonates influence the formation of aragonite in shell. Some support to this idea is found in the fact that in vitro the crystallization of aragonite is facilitated by strontium and lead salts. This observation made by Prenant (1924) apparently influenced Trueman's (1942) hypothesis that strontium, magnesium, and probably other salts found in living mollusks influence the crystallization of aragonite.

That there may be some correlation between the predominance of the particular mineralogical form of calcium carbonate and the temperature of the surrounding water has recently been suggested by some geologists. Through quantitative X-ray analysis of shells they have demonstrated that in certain polyclad worms (Serpulidae) and in some gastropods and pelecypods (*Mytilus*, *Volsella*, *Pinctada*, *Anomia*, and others) the concentration of aragonite in shells increases with increasing temperatures (Epstein and Lowenstam, 1953; Lowenstam, 1954). In *Mytilus*, for instance, only the shells of warm water species are composed entirely of aragonite, whereas those from colder waters contain varying amounts of both calcite and aragonite. This interesting ecological observation does not, however, provide a clue to the nature of the biochemical processes which control the predominance of one or another crystallization system.

RATE OF CALCIFICATION

The calcification rate of the left valve of *C. virginica* is significantly higher than that of the right one, as can be readily seen by examining newly formed shells. The calcareous material deposited by the left mantle is thicker and heavier than that deposited during the same time by the right mantle (Galtsoff, 1955). I made the following observations on shell growth rate of adult *C. virginica*. After the new growth of shell along the valve edge was carefully removed the oysters were placed in tanks abundantly supplied with running sea water. About 2 months later the areas of newly deposited shells on each valve were measured with a planimeter, carefully removed from the shell, rinsed in distilled water, dried in air, and weighed. The results are summarized in table 13. In every case the amount of calcified material deposited over a unit of area was considerably greater on the left

TABLE 13.—*Areas of new growth and rate of deposition of shell material by* C. virginica *in mg. per day per cm.*^{2} *during April to June 1954, Woods Hole, Mass.*

Oysters	Area of new shell	Weight per cm.^{2}	Deposition per cm.^{2} pay day	Days under observation	Ratio weight of left to weight of right valve
Five-year-old, Narragansett Bay	*Cm.*^{2}	*Mg.*	*Mg.*	*No.*	
Left valve	5.80	156.0	2.8	55	2.6
Right valve	5.16	59.3	1.1		
Adult, Narragansett Bay					
Left valve	7.1	123.0	1.8	68	6.2
Right valve	7.7	19.9	0.3		
Adult, Narragansett Bay					
Left valve	6.1	74.2	1.09	68	2.9
Right valve	8.8	25.5	0.37		
Two-year-old, New Hampshire					
Left valve	3.68	163.8	2.98	55	3.2
Right valve	4.20	52.0	0.95		
Very old, New Hampshire					
Left valve	6.83	71.2	1.3	55	2.2
Right valve	7.35	33.0	0.6		

valve (lower) than on the right one (upper), the difference varying from 2.2 to 6.2 times.

The rate of deposition of calcified material by the surface of the mantle may also be studied by inserting between the mantle and shell small pieces of plastic or other nontoxic material of known area and weight. Results obtained with this method vary greatly. Observations made on 16 adult oysters at Woods Hole during the period of August 9 to 20, 1953, show that in 15 oysters the daily rate of shell deposition per square centimeter varied from 0.4 to 2.1 mg. One oyster deposited 14.2 mg. in 2 days or 7.1 mg. per day. The amounts of shell material deposited by 20 Narragansett Bay oysters kept in laboratory tanks for 68 days during the period of April to June varied from 0.1 to 0.79 mg. of shell substance per day $cm.^2$. In some of these oysters the presence of the plastic material induced pathological conditions which resulted in the formation of leathery capsules similar to the blisters frequently found on the inside of shells near the adductor muscle. The formation of such blisters was accompanied by deposition of calcite greatly in excess of the rate of calcification under normal conditions.

Seasonal variation in rate of shell deposition over the inner surface of the valves was also studied, using 20 adult oysters for each set of determinations. Observations were continuous from June 1954 until the end of February 1956. To avoid possible injury to the mantle while introducing pieces of plastic, the oysters were fully narcotized in magnesium sulfate solution and insertions made when the mantle was completely relaxed and did not respond to touch. Thin sheets of plastic were cut into rectangular pieces 0.5 $cm.^2$ in area and weighed before inserting them under the mantle, their weight varying from 5.5 to 6.0 mg. Some of the pieces introduced were ejected by the oysters, but losses were minimized when the insertion was made under full narcosis. The treated oyster was then marked and placed on its left valve in a large tray supplied with running sea water. The temperature of the water was recorded twice a day. Each set of 20 oysters was kept in water as long as the seasonal rise or fall of water temperature did not exceed 2.5° C.

To obtain measurable quantities of shell deposits the pieces of plastic were left inside the oysters for a longer time in winter and in August, after the completion of spawning, than during the rest of the year. The number of days the oysters with inserted pieces were left undisturbed varied as follows: from 10 to 16 days in April to July; from 25 to 30 days in August; from 13 to 18 days in September to November; for 30 days in December; and 70 days in January to March. Observations were continued for 14 months. No shell was formed in January to March except in a few oysters in which the mantle was injured during insertion. These samples were not included in the data plotted in fig. 102. Laboratory observations showed that shell opening and feeding of the oysters at Woods Hole are as a rule temporarily reduced after the discharge of sex products which takes place late in July and early in August. Unequal time intervals in observing shell deposition do not affect the validity of the results since the rates of shell formation shown in figure 102 are expressed as weights of shell deposited per $cm.^2$ in 1 day.

At the end of each period the oysters were removed, the pieces of plastic recovered, rinsed in distilled water, dried at 55° C., and weighed. The results summarized in figure 102 are shown as medians (Md.) of the rate of shell deposition per $cm.^2$ per day, and as lower (Q_1) and upper (Q_3) quartiles.

The curves show two periods of accelerated shell growth in Woods Hole water, one in May to June and another in October, and no shell growth during winter from December to the end of April when the temperature of the water varied between 1° and 2° C. These observations are in agreement with many field data and with the experiences of practical oyster growers of the North Atlantic states, who found that oysters grow more rapidly in the spring and in the autumn and cease to grow when the water temperature drops to about 5° C. The relatively low rate of shell deposition during the summer is attributable to the inhibitory effect of fully developed gonads. Observations frequently made in the Woods Hole laboratory show that shell growth in the winter will begin within 24 hours after the transfer of oysters from the harbor to much warmer sea water in the laboratory.

Under normal conditions no shell is deposited in winter. In several instances, however, large amounts of shell material were secreted over an area of the mantle which was apparently injured by the insertion of plastic. One of these cases is shown in figure 103. In this oyster a heavy pocket of shell material was deposited on the valve over

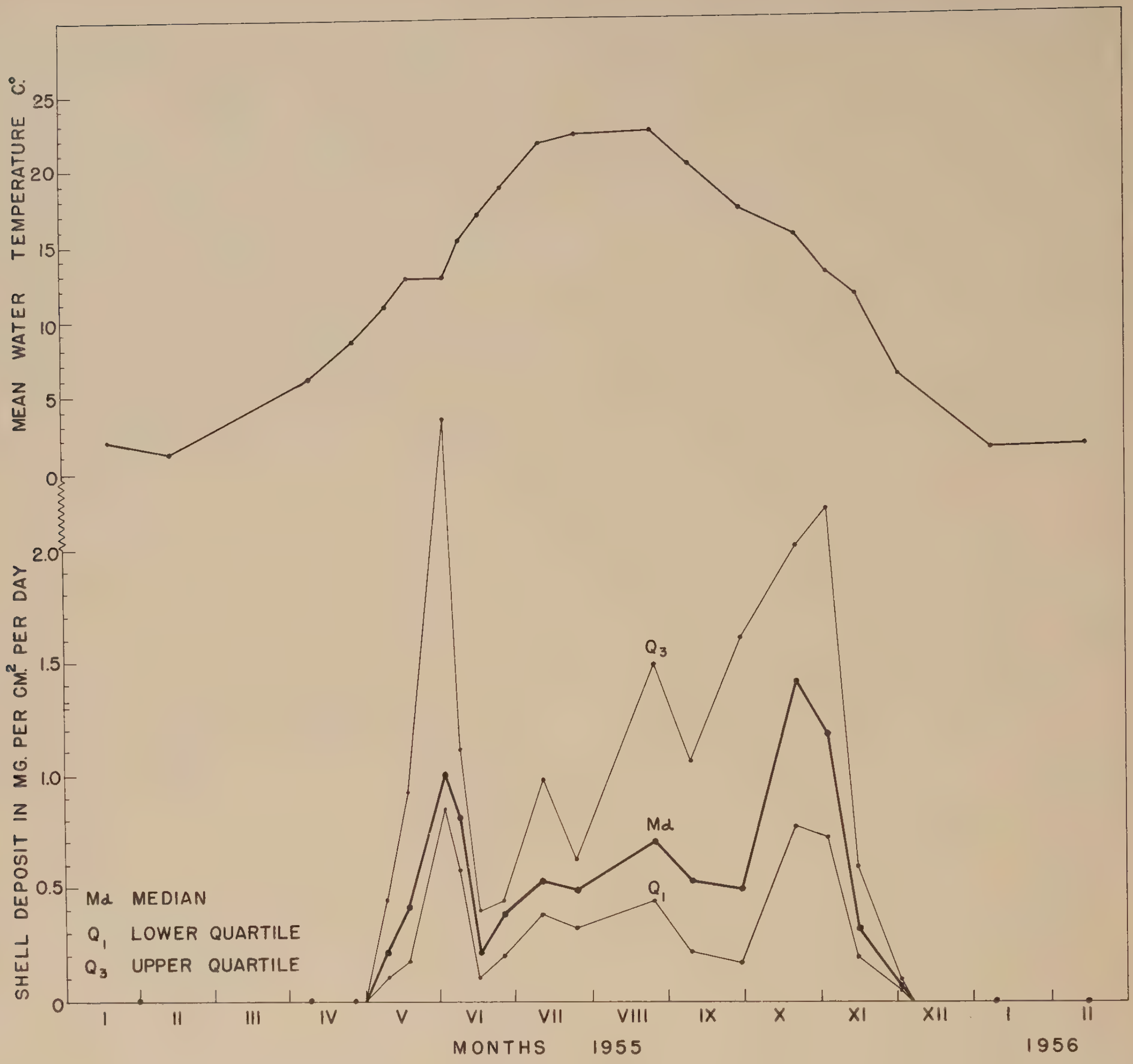

FIGURE 102.—Seasonal changes in the rate of deposition of shell material over the inner surfaces of left (lower) valves.

the area occupied by a piece of plastic, and a shell ridge was formed along the edge of the mantle, which was withdrawn a considerable distance back from its normal position. It can be deduced from these observations that injury to the mantle stimulates the shell secretion and that deposition may take place even at low temperatures when normal shell growth is inhibited. This would indicate that the enzymatic system involved in shell deposition is always present and may become active in response to pathological conditions in spite of the inhibitory effect of winter temperatures.

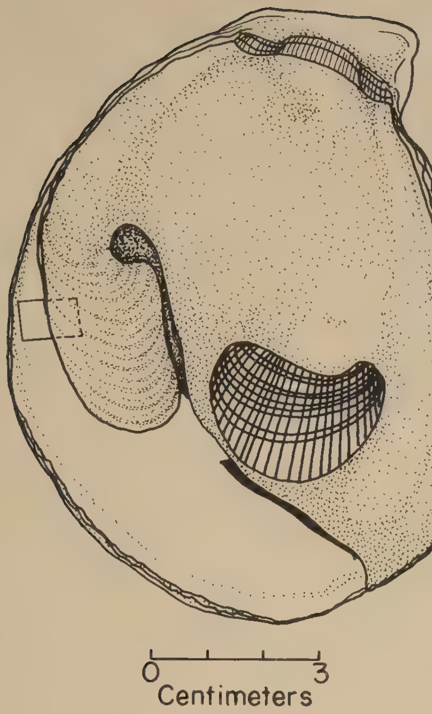

Figure 103.—Abnormal deposition of shell material along the edge of the mantle (black line) and over the piece of plastic quadrangle, which was completely encapsulated in a pocket of newly secreted shell. Mantle is shown by stippled area. Winter observation at Woods Hole.

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CHAPTER VI

THE LABIAL PALPS

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ANATOMY

The four soft flaps which lie at the anterodorsal side of the body under the mantle hood are the labial palps. Their triangular members are attached by their broad bases to the visceral mass and have slightly curved margins which extend ventrally to the point where they touch the free edges of the gills (fig. 104).

The two pairs of palps, one on each side, are joined together into a single unit which serves primarily for final sorting of food particles and for the delivery of food to the mouth. Each pair consists of one external and one internal palp (r.i.p.; r.o.p.). The two external palps join together above the mouth (m.) where they form the upper lip (u.l.); the two internal palps are united below the mouth into a lower lip (1.1.). As a result of this arrangement the mouth is an irregularly shaped, narrow, curved slit. Both lips are arched; the lower one is shorter, and its edge is thicker than that of the upper lip.

At the central junction of the two internal palps there is a median gutter which leads to the center of the lower lip. The two lateral gutters (l.g.) formed on each side where the external palp meets its opposing internal member are the principal paths by which the food is conveyed to the corners of the mouth (fig. 104). The surface is smooth along the outer part of the external palps and on the inner palps along the median plane, where the palps meet; along the lateral gutters, both have a striated appearance due to numerous ridges and grooves which empty into the corresponding gutter. This arrangement is significant for an effective sorting of food.

HISTOLOGY

Each labial palp consists of a layer of connective tissue covered on both sides by columnar ciliated epithelium set on a basement membrane. These are made of large vesicular cells of the type found in the mantle (fig. 105, c.c.). Within the body of a palp there are many blood vessels and blood spaces or sinuses. These spaces are splits or interstices between the connective tissue cells and have no lining or wall. The longitudinal and transverse muscle fibers are numerous but not as well developed as they are in the mantle.

The smooth sides of the palps are covered with small epithelial cells, about 5 to 6μ long, with minute cilia not exceeding 3μ in length. Awati and Rai (1931) maintain that in *O. cucullata* only some of the epithelial cells of this surface are ciliated. They suggest that the ciliated cells have sensory function but present no evidence to support this view. The absence of cilia on some of the cells of this layer may be due to their destruction during the processing of tissue.

In *C. virginica* the epithelium of the smooth surface of the palp consists of almost cubical cells with relatively large nuclei and small cilia (fig. 105, c.ep.). Cell boundaries are indistinct, the cells themselves are crowded and compressed, and there is a very thin and transparent cuticle on the periphery. In the subepithelial layer large eosinophilic cells (e.c.) and mucous cells (m.c.) are very abundant. The mucous cells are frequently pear-shaped, but their appearance varies, depending on the amount of secretion they contain. Their length is between 25 and 30 μ. Wherever the secretion of mucus is least, the outer surface of the palp is slightly ruffled, as shown on the right side of figure 106, representing a longitudinal section of the palp viewed at low magnification.

The inner or ridged surfaces of the palps present a different picture. The entire surface is folded into deep ridges and grooves. In figure 106 the ridges are about 0.3 mm. high along the central axis. The ciliated cells of the ridged surface are slender, cylindrical, and tightly packed, with small, round nuclei. They form a layer varying from 40 to 60 μ in thickness. The difference in

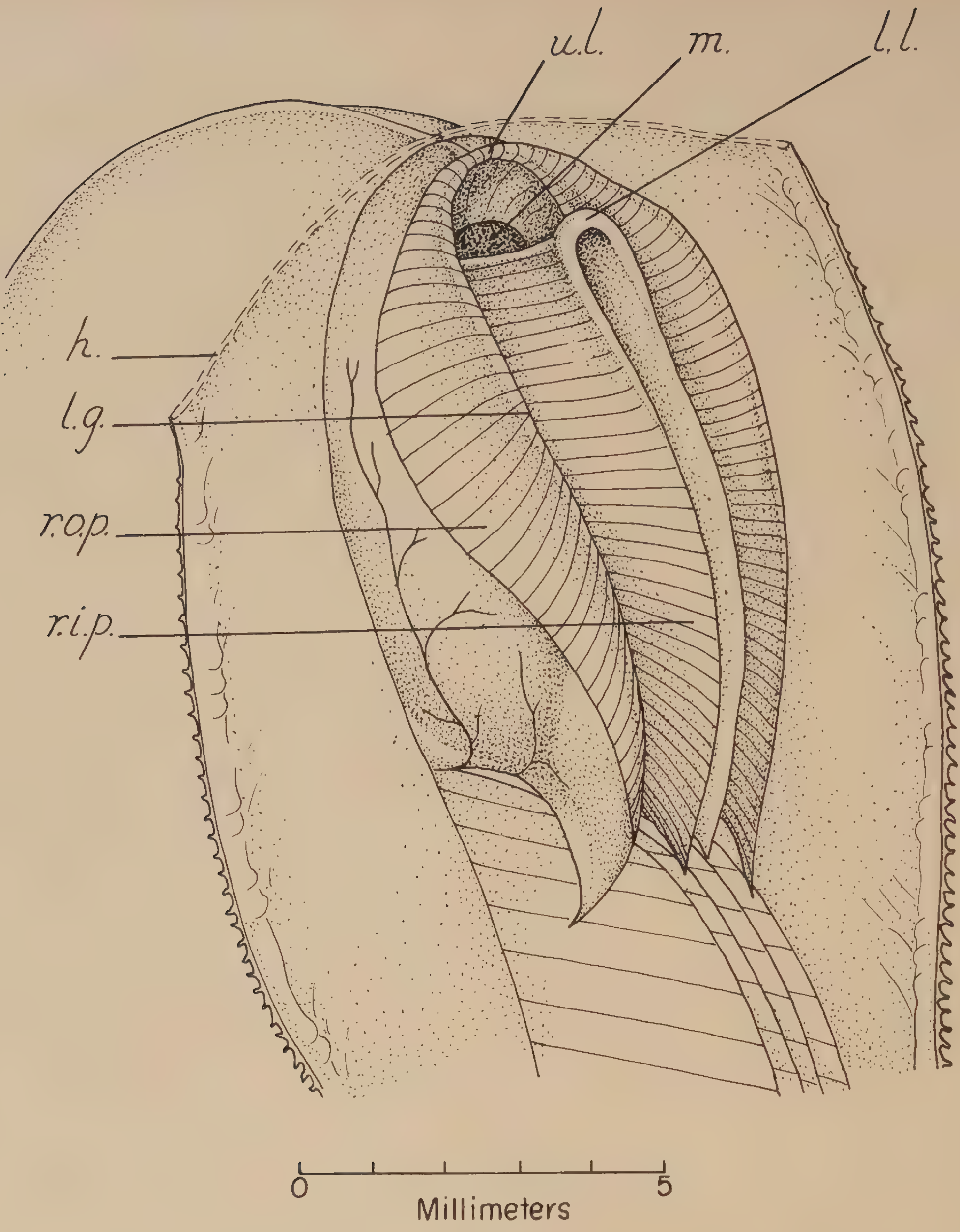

FIGURE 104.—Labial palps of *C. virginica*. The mantle hood is cut off at the middle, and the sides are pulled aside to expose the lips and the mouth. Drawn from life. h.—hood; l.g.—lateral gutter; 1.1.—lower lip; m.—mouth; r.i.p.—right inner palp; r.o.p.—right outer palp; u.l.—upper lip.

the shape and size of the cells of the two sides of the palp is clearly seen in this figure.

Thin and transparent cuticle covers the epithelium of the ridged surfaces. The cilia are robust, ranging in length from 8 μ in the grooves to 20 μ on the tips of the ridges, with the longest cilia found near the free edge of the palps. Mucous cells are present but are less abundant than on the smooth side.

The ridges themselves are set at an acute angle to the surface of the palp and recline toward its free edge. There is a noticeable difference in the epithelium of the two sides of the same ridge. The cells lining the sides which face the mouth (lower sides of ridges in figure 106) have longer cilia, and the entire epithelium is slightly ruffled. On the opposite sides the ciliated cells are uniform and have smaller cilia. This difference appears on a tangential section of the palp shown in figure 107.

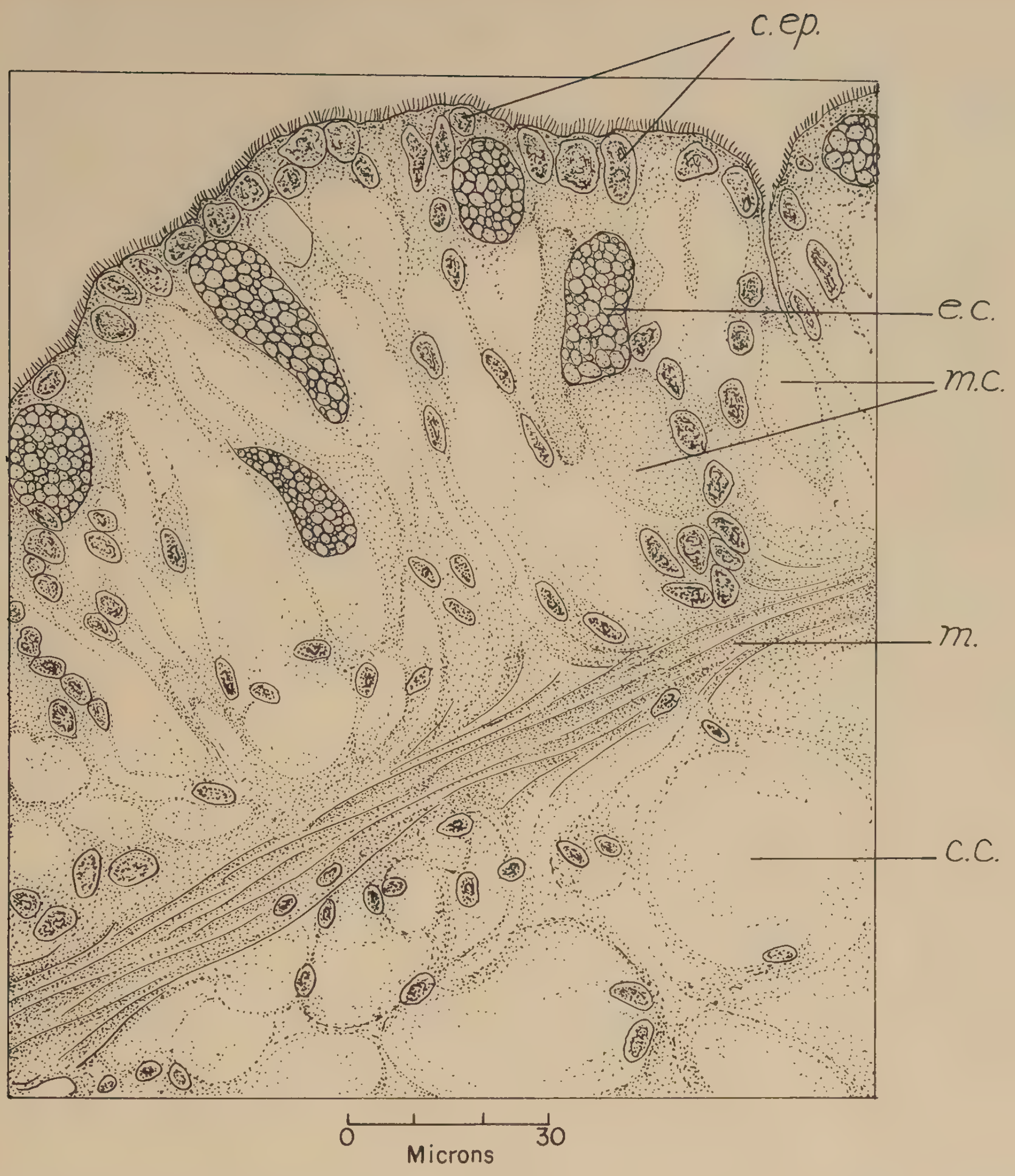

FIGURE 105.—Cross section of the smooth side of labial palp of *C. virginica*. c.ep.—cubical ciliated epithelium; e.c.—large eosinophilic cell; m.c.—mucous cell; m.—muscle fibers; c.c.—vesicular cells of connective tissue. Note the the infiltration of subepithelial layer by mucus and invasion of blood cells. Bouïn, hematoxylin-eosin.

The epithelium rests on a thin basal membrane consisting of connective tissue fibers and muscles. The core of the ridge is made of delicate connective tissue with occasional large vesicular cells containing eosinophilic granules. The description of microscopical structure of the palps given above is in agreement with the observations made on various bivalves by previous workers (Leenhardt, 1926; List, 1902; Siebert, 1913; Thiele, 1886; and Wallengren, 1905a, 1905b).

The palps are well supplied with blood delivered through the anterior aorta, pallial artery, and short tentacular artery. The velar artery branches off from the short tentacular artery and runs the entire length of the palp, giving off numerous ramifications. According to Schwaneke's observation (1913) on *Anodonta*, the blood from the palp is returned to the heart by the way of the mantle.

The palps are innervated by the nerve emerging from the cerebral ganglion and entering the anterior end of the junction between the paired

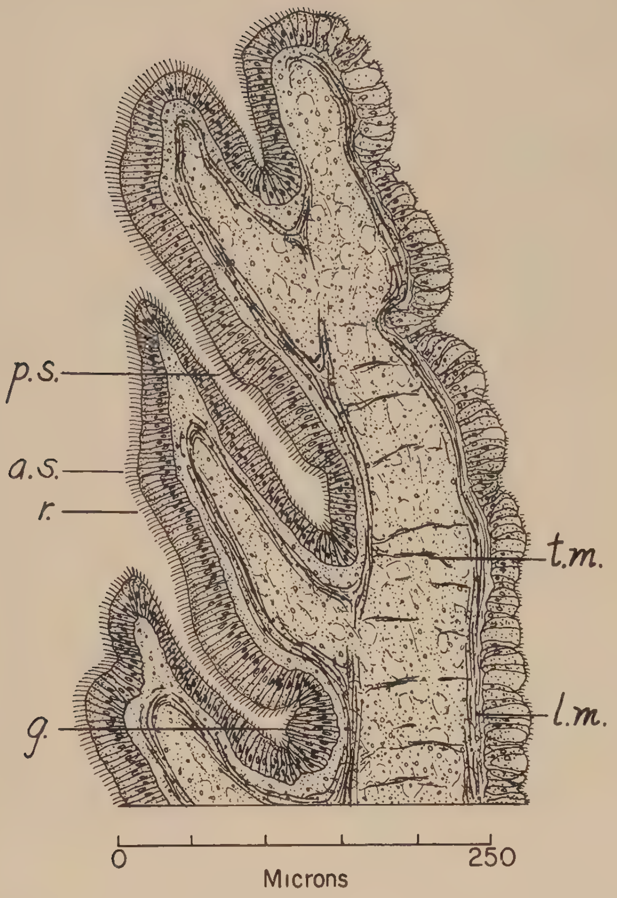

FIGURE 106.—Longitudinal section of a portion of the right external palp. The right (smooth) side is only slightly ruffled. The left side is folded and forms deep ridges and grooves. Bouin, hematoxylin-eosin. a.s.—anterior slope of the ridge; g.—grooves; l.m.—longitudinal muscles; p.s.—posterior slope of the ridge; r.—ridge covered with ciliated epithelium; t.m.—transverse muscles.

lobes. A nerve net was described in the palps of *Anodonta* (Matthews, 1928). I was not able to demonstrate its presence in the oyster palps.

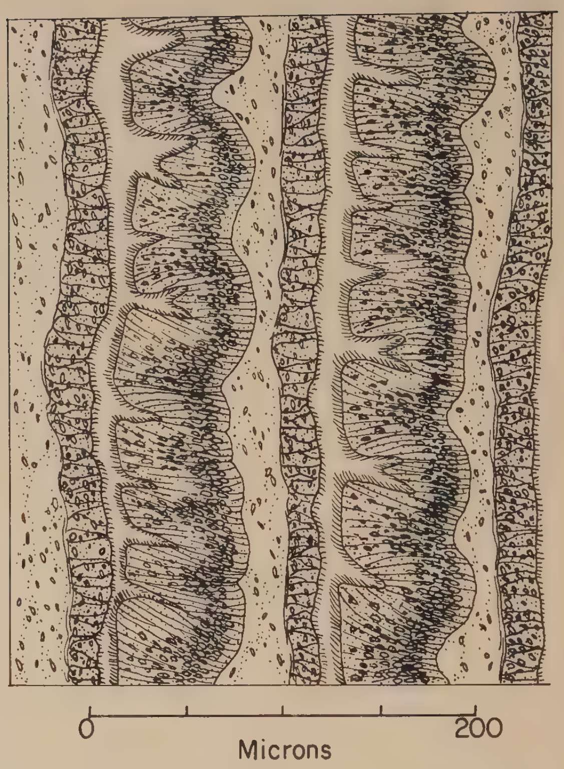

FIGURE 107.—Tangential section of the inner surface of labial palp of *C. virginica* made through two and a half ridges. The ruffled sides of the ridges are directed anteriorly toward the mouth. Bouin, hematoxylin-eosin.

DIRECTION OF CILIARY CURRENTS

The most conspicous features of the palps are the abundance of mucous cells on their smooth sides and the powerful ciliated layer covering the ridges of the inner sides. The idea that labial palps of bivalves represent a sorting apparatus was clearly stated by Coupin (1893), who observed that in *Mytilus*, *Cardium*, and *Pholas* the more voluminous particles are discarded by the palps while the finest ones are delivered to the mouth. He concluded that the principal function of the palps of *Pholas* is to prevent the bulky material from reaching the digestive tract.

The sorting of food is accomplished by a complex system of ciliary currents along the surfaces of the palps. With reference to *C. virginica*, Kellogg (1915) states that the smooth sides of the two internal palps facing each other direct the food particles backward to the tips of the palps. This statement is only partially correct.

In describing the feeding habits of fresh-water mussels Allen (1914) noticed that the ridges of the inner surfaces of the palps normally overlap one another in a reclining position. This is also the usual position of the ridges in *C. virginica* (figs. 106 and 108). On the anterior slope of each ridge (fig. 106, a.s.) the currents are directed toward the free end of the palp; the currents of the posterior slope (p.s.) lead toward the mouth. Thus the final direction of the movement of a particle along the surface of the palp depends on the position of the ridges. Allen thinks that, as long as no adverse stimuli are received, the particles which lie between the palps pass on forward

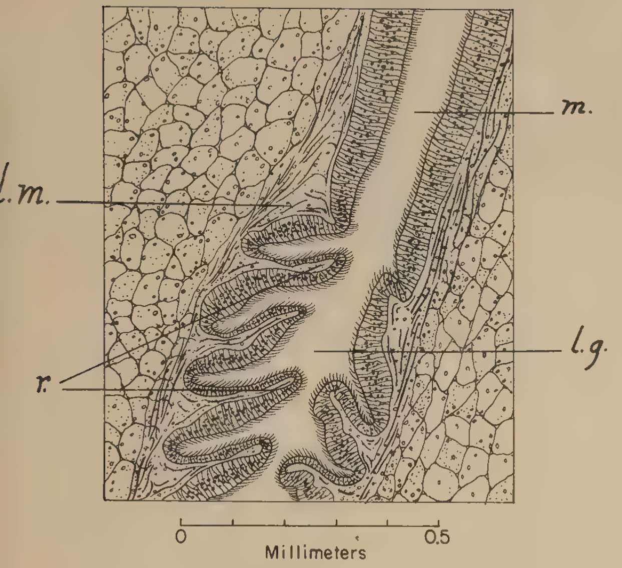

FIGURE 108.—Longitudinal section of the palps along the lateral groove at the place of entrance to the mouth. l.g.—lateral groove; l.m.—longitudinal muscles; m.—mouth; r.—ridges of the inner sides of palps. Bouin, hematoxylin-eosin.

from one ridge to another and eventually reach the mouth. In case of irritation the reflex erection of the ridges brings uppermost the cilia leading backward, and the particles on the surface of the palp are pushed from summit to summit to the edge of the palps and discarded. It is not clear from Allen's description whether his explanation is based on observations or on an assumption that the ridges are capable of changing their position in response to stimulation.

During observations of the feeding of an oyster spat less than 3 mm. long and attached to a glass slide, Nelson (1923) found that the filaments of the palps narcotized in magnesium sulfate solution lose their power of erection with the result that large masses of material passing over the filaments accumulated near the mouth and blocked it. This effect may have been caused by the inhibition of ciliary motion as well as by the suppression of muscular contraction.

The "filaments" of the palps of the spat observed by Nelson undoubtedly correspond to the ridges of the inner surfaces of the palps of adult oysters. The theory of sorting out of the material advanced by Allen and accepted by Nelson implies that the change in the direction of movement of the particles toward the mouth or away from it is controlled by the erection of the ridges. With a microscope I examined the palps of oysters intact except for the removal of one valve and was not able to observe changes in the position of the ridges. Similarly, no erection of ridges was noticed on the excised palps which were kept in sea water and examined with high power. The ciliary currents remained, as a rule, undisturbed for many hours.

Yonge (1926) states that in *O. edulis* "there are no muscles within the folds such as could cause it to contract downwards." This statement cannot be confirmed by my observations on *C. virginica.* In the palps of the American osyter the longitudinal muscles are clearly visible (figs. 106 and 108, l.m.). They extend along both sides of a ridge from its base to the very tip. The contraction of muscle fibers on one side of the ridge presumably may change the position of the ridge and make it stand at right angles to the surface of the palp. There is no evidence that this actually happens.

Churchill and Lewis (1924) arrived at the conclusion that in fresh-water mussels the cilia covering the upper portions of the ridge beat forward (toward the mouth) while in the deeper part of the groove between the ridges they beat in the opposite direction. They also make a generalization that "the palps appear to be a mechanism for reducing the quantity of material to an amount that can be handled by the mouth." According to these authors the two sides of the palps perform distinctly different functions. In fresh-water mussels the ciliary currents along the outer (smooth) surfaces remove the particles from the mantle chamber, while on the ridged surfaces the currents are directed toward the mouth.

The currents along the labial palps of *C. virginica* are, however, more complex and somewhat different from those described for fresh-water mussels. To observe these currents I removed one valve, dissected the mantle hood, and pushed apart its sides to expose the entire surface of the palps. I placed the oyster in sea water in a shallow tray and observed it with a low-power microscope under strong illumination directed at an angle of about 20 degrees to the surface. Suspensions of colloidal carbon or finely powdered willemite in sea water were used to flood the surface, and the distribution of the brilliantly fluorescent willemite particles was observed under ultraviolet light. A series of pencil diagrams outlining the positions of the palps were prepared in advance and were used to mark the direction

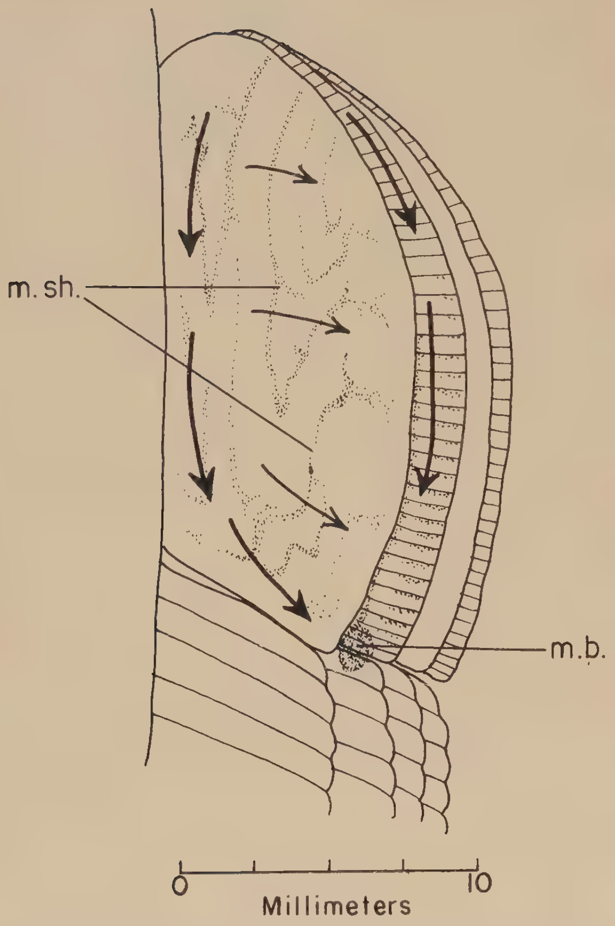

Figure 109.—Ciliary currents along the outer surface of the right external palp of *C. virginica.* Dark streaks (dotted areas) represent the distribution of mucus strands with black particles entangled in them 2 or 3 minutes after the surface was flooded with suspension of colloidal carbon. Large arrows indicate the direction of principal currents; small arrows show the secondary currents directed toward the free edge of the palp. The material entangled in a slimy ball (m.b.) is discarded at the corner of the palp. m.sh.—mucus sheet. Drawn from life.

of principal currents as they were observed immediately upon the addition of the suspended material. Figures 109 through 112 summarize the results of many observations on several large oysters.

The palps are usually totally covered with a sheet of mucus, but the secretion of the mucus is greatest on the outer surfaces of the external palps. Numerous mucous glands under the surface epithelium (fig. 105) are easily stimulated by the slightest irritation and begin to discharge large amounts of mucus as soon as their surface is flooded with water containing suspended particles. Figure 109 shows the two systems of currents, both directed away from the mouth. A strong current along the base and an equally powerful current along the free edge run toward the lower free corner of the palp. Weaker currents (small arrows) are directed across the surface from the base of the palp to its free edge where the particles cleared from the area are picked up by the strong ciliary current at the periphery and are carried to the corner of the palp touching the edge of the gill (m.). Here a strong eddy is formed, probably as a result of combined downward currents of the palp and of the ciliary epithelium of the gills. The particles discarded from the palps rotate in this area until a fairly large ball measuring about 0.5 cm. is formed and

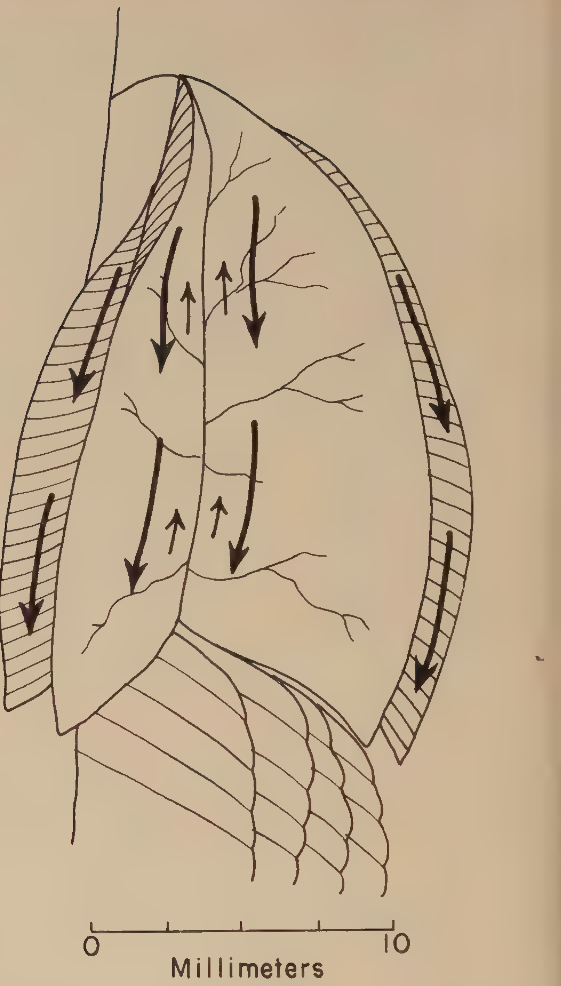

Figure 110.—Principal ciliary currents along the smooth surfaces of internal palps, *C. virginica.* Small arrows indicate the weak countercurrent directed toward the mouth at the bottom of the central gutter. Drawn from life.

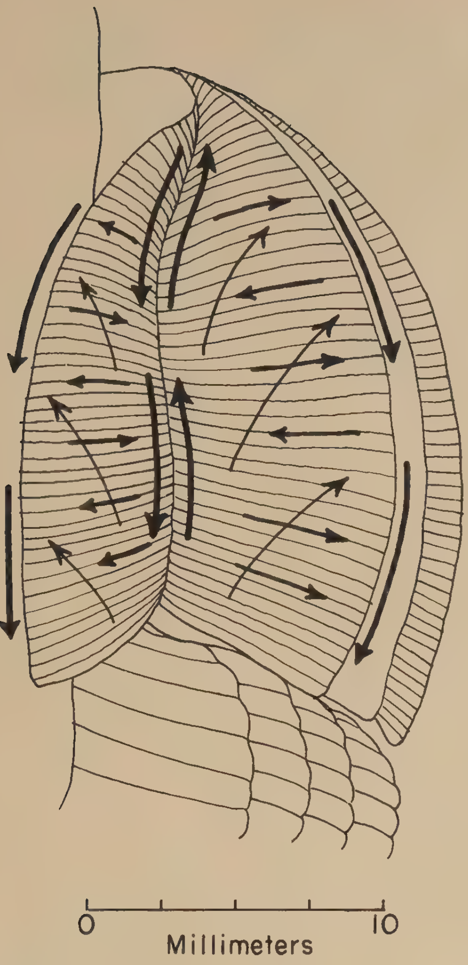

FIGURE 111.—System of currents along the ridged surfaces of the palps, *C. virginica*. Currents along the edges of the palps and at the bottom of lateral gutter are shown in heavy long arrows. The current and countercurrent along the ridges are shown by short arrows. Long narrow arrows denote the movements of particles across the palp toward its free edge. Drawn from life.

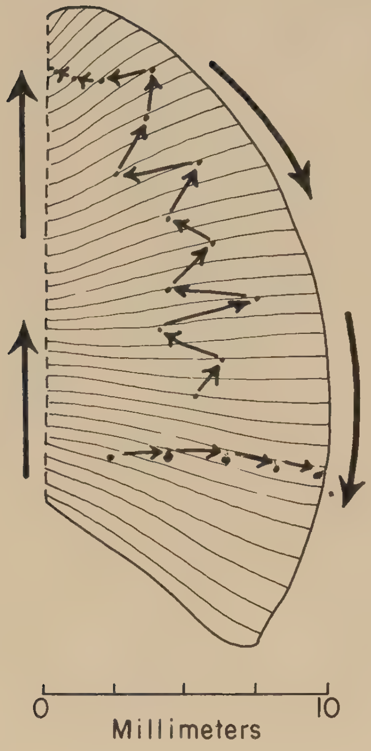

FIGURE 112.—The paths of two particles across the ridged surface of a palp (small arrows), *C. virginica*. Small particle at the lower part of the diagram fell between the two ridges into the furrow and was carried away by the current along the edge. A slightly large particle (upper half of drawing) settled on the top of a ridge and was kicked from one crest to another until it reached the bottom current of the lateral gutter and was pushed toward the mouth. Drawn from life.

the mass is dropped to the surface of the mantle and discarded through the principal discharge area (fig. 90). The currents along the smooth surfaces of the two internal palps (fig. 110) are directed primarily away from the mouth and toward the free edges along the entire length of the palps. There is a much weaker countercurrent along the central gutter. The material brought by this current reaches the lower lip and may be pushed into the mouth (fig. 113). Some of the particles which reach the rounded rim of the lower lip drop back to the surface of the palp and are carried away by the principal currents.

The system of currents on the ridged surfaces of the palps is very complex (fig. 111). There are two major and opposing currents along the lateral groove. The bottom current runs toward the mouth; at a slightly higher level (heavy long arrows) the currents run in the opposite direction. Equally strong currents directed away from the mouth run along the free edge of the palps.

There are at least three major currents along the inner surfaces of the palps: (a) from the base across the palp to the edge, (b) in an opposite direction from the edge to the base (fig. 111, short heavy arrows), and (c) slightly weaker currents from the central part of the palps at about 45 degrees to the edge (long slender arrows).

A particle moving along the ridged surface of the palp may follow a very irregular path. Figure 112 diagrams the route of two particles which both were pushed away from the surface. One particle (upper part of fig. 112) settled on the edge of a ridge and was pushed by the uppermost cilia from the top of one ridge to the others until it reached

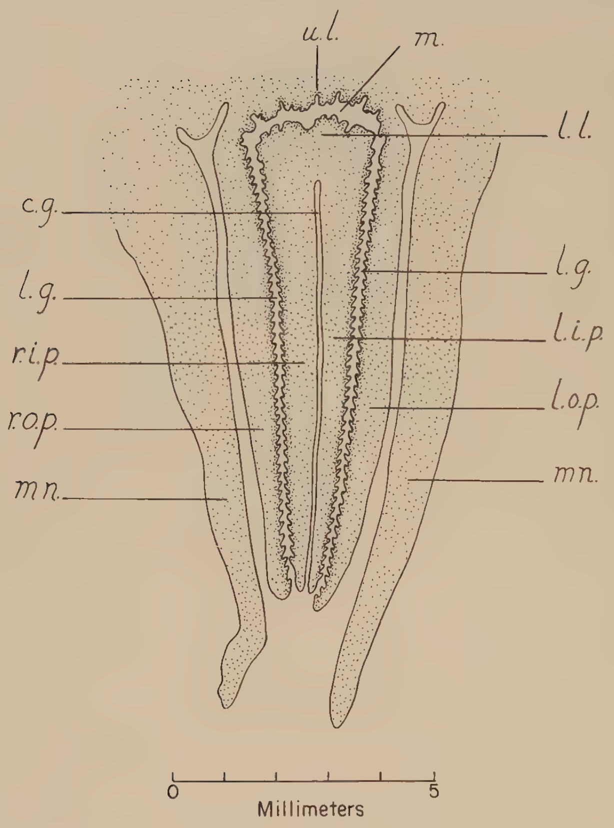

FIGURE 113.—Tangential section across the mouth and palps. Semidiagrammatic. c.g.—central gutter; l.g.—lateral gutter; l.l.—lower lip; l.i.p.—left inner palp; l.o.p.—left outer palp; m.—mouth; mn.—mantle; r.i.p.—right inner palp; r.o.p.—right outer palp; u.l.—upper lip. Formalin and hematoxylin.

the base and was carried toward the mouth. Another particle (lower part of fig. 112) fell into the furrow; several times it was kicked over two or three ridges, but it always fell to the bottom of a furrow and finally was carried toward the edge of the palp and discarded (heavy arrow). Frequently the medium-sized particles entangled in mucus are caught in an eddy between the ridges and begin to rotate in a turbulent current. Additional particles are entangled, and as the ball grows it is kicked out and caught by the current along the edge. There was no evidence of the reversal of ciliary motion and the pattern of currents described above was maintained throughout the periods of observations.

An effective sorting mechanism, which prevents all but the smallest particles from reaching the mouth, is provided by the system of ciliary movement. The main supply of food is delivered by the lateral gutters (fig. 113), which lead directly into the corners of the mouth. Only a small amount of food reaches the mouth via the central gutter where it must be pushed over the edge of the lower lip. Observations of the ciliary motion of the palps give one the impression that the number of food particles delivered to the mouth constitutes only a small fraction of the total quantity which reaches the palps. It appears that the principal function of the palps is to eliminate a large part of food gathered in feeding and allow only a very small portion of it to reach the digestive tract. The system of currents and countercurrents observed in the palps of *C. virginica* is much more complex than that described by Yonge (1926) for the palps of O. *edulis*.

Mucus plays an important role in the sorting of food. The discharge of mucus is directly proportional to the amount of particles which settle on the surface of the palps. The sheet of mucus is pulled over the ridges of the palps by the action of the more powerful cilia at the tops of the ridges. The smaller cilia of the grooves may not be in direct contact with the mucus sheet above them and beat in a different direction. Only the smallest particles, free of mucus, are moved directly by the cilia.

A study of the ciliary currents of the palps suggests that the food-sorting mechanism of the oyster is designed for continuous feeding in water containing a low concentration of minute food organisms. Under such conditions only a limited amount of mucus is used for sorting out the suitable forms. The question of whether the oyster is capable of selecting its food on some other basis than size has not yet been adequately studied.

REACTION TO STIMULI

The palps respond both to mechanical stimulation and to the chemical stimulation of weak acids, solutions of mineral salts, etc. If a weak mechanical stimulus is applied to the edge of the palp, the affected portion contracts, making a shallow indentation which is proportional in size to the intensity of the stimulus. Strong mechanical stimulus or application of such irritating solutions as weak hydrochloric acid, ethyl alcohol, adrenaline, etc., provoke general contraction of the palps and gradual curling up of their edges. The external palps bend to the outside, while the internal palps curl in the opposite direction and seal the access to the central gutter (fig. 114). Autonomous responses of the labial palps of *Ano-*

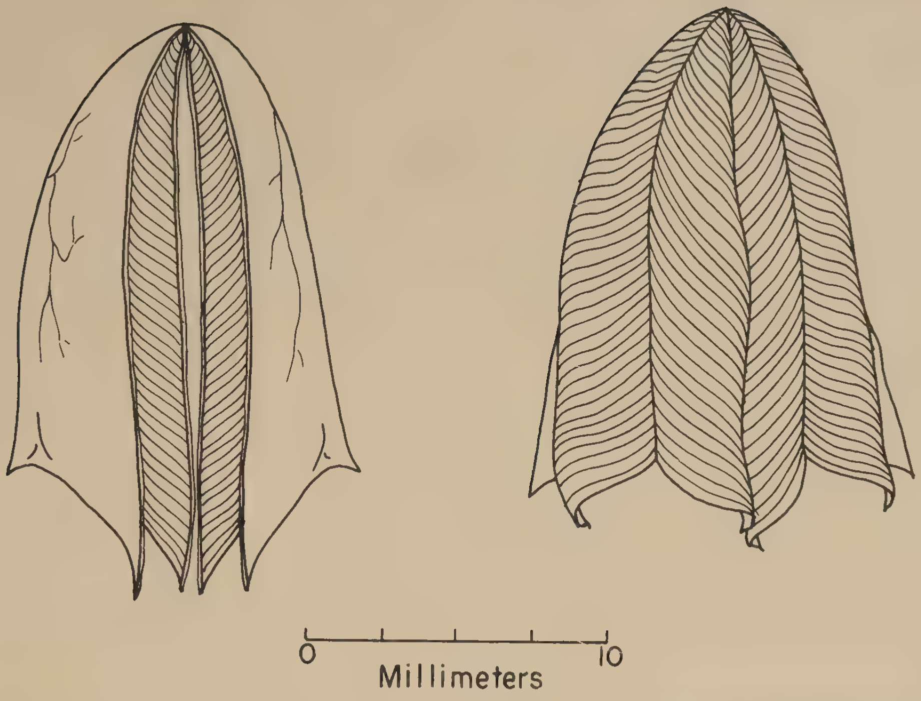

FIGURE 114.—Labial palps. Left—normal position. Right—position taken after mechanical stimulation. Drawn from life. Slightly enlarged.

donta were studied by Cobb (1918), who found that curling can be evoked by a jet of warm water (54° C.), a beam of sunlight, faradic current, and by a number of chemicals. His conclusion that chemical stimulation is more effective than are the mechanical stimuli in provoking a response could not be verified. While it is easy to determine the effectiveness of various concentrations of chemicals or to register the response to mechanical stimuli, a direct comparison between the intensities of chemical vs. mechanical stimulation is impossible without having a common standard for reference.

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CHAPTER VII

THE GILLS

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The gills of the oyster and other bivalves perform several important functions. They play a major part in respiration to which the mantle contributes a minor share. They maintain a steady current, filter the water, and collect food particles which are sorted and separated from detritus and other materials in suspension. They serve for the dispersal of sex cells at the time of spawning, and are used for the incubation of fertilized eggs in the larviparous species. The effectiveness of these functions is dependent on coordinated performance of the gill apparatus and on the contractions of the adductor muscle.

ANATOMY OF THE GILLS

Within the class of bivalves the structure of the gills varies in an increasingly complex series of modifications. The simplest of these is one pair of plumlike single gills or ctenidia with two rows of flattened filaments on each gill. This primitive type, present in the order Protobranchia (fig. 115, A), is found in *Nucula*, *Yoldia*, *Leda*, *Solenomya*, and others. More complex structure (fig. 115, B) occurs in the ark shells (Arcidae), scallops (Pectinidae), oysters (Ostreidae), sea mussels (Mytilidae), and other families of Filibranchia. This type is characterized by long and slender filaments kept in place by patches of interlocking cilia. In some of the bivalves of this group, including edible oysters, the gill lamellae are plaited into vertical folds and the reflected plates of the gills are completely united with the mantle and visceral mass. These were formerly designated as a separate order of Pseudolamellibranchia.

The highest degree of complexity is found in the gills of fresh-water mussels (Unionidae), cockles (Cardiidae), clams (Veneridae), and many other mollusks of the order Eulamellibranchia. In these bivalves the lamellae are joined by bars of connective tissue, the filaments are firmly connected by vascular junctions, and the entire gill has the appearance of a perforated, leaflike organ (fig. 115, C).

In the order Septibranchia (*Poromya*, *Cuspidaria*) the gills are degenerate. They are modified into perforated, muscular partitions between the two pallial chambers, and the gill filaments are greatly reduced (fig. 115, D).

The oyster gills consist of four folds (demibranchs or plates) of tissue suspended from the visceral mass. Two folds on each side of the body arise from a common ridge or gill axis, which is composed of connective tissue and muscles (fig. 73, g.m.). In a cross section made at right angles to the axis of the gill, each demibranch is V-shaped, with the two branches of the V forming its ascending and descending lamellae. The descending lamella arises from the gill axis; its opposite number is the ascending lamella. The two outer ascending lamellae, one on each side of the body, are fused with the mantle. The two inner demibranchs are joined together at

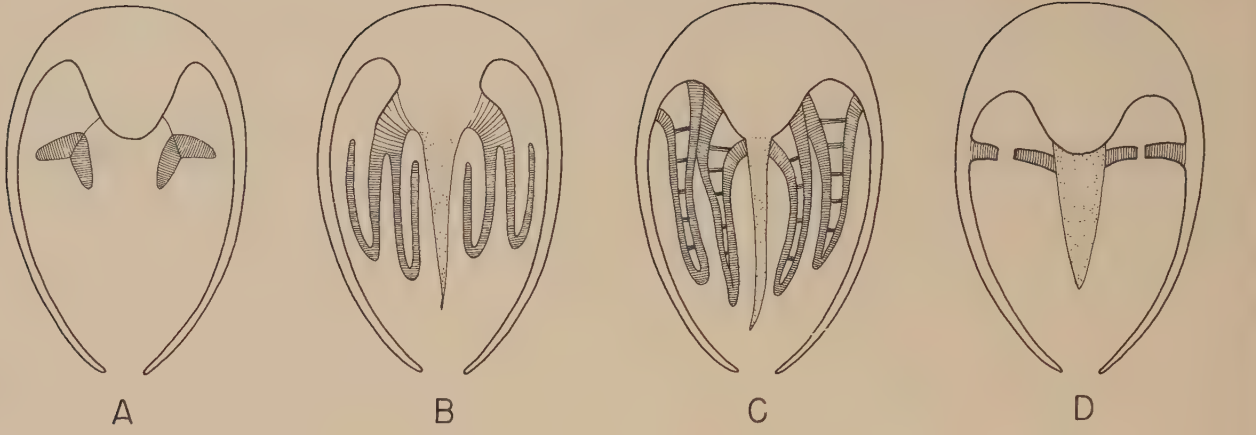

FIGURE 115.—Diagram of the four types of bivalve gills according to A. Lang (1900). A—Protobranchia type (*Nucula, Yoldia, Leda,* and others); B—Filibranchia type (Pectinidae, Ostreidae, Mytilidae, and others); C—Eulamellibranchia type (Unionidae, Cardiidae, Veneridae, and others); D—Septibranchia type (*Poromya, Cuspidaria*).

the central axis of the gills under the common afferent vein (fig. 73, c. af. v.). The section across all four demibranchs resembles two W letters joined together at the center.

Along the entire length of the gill runs the interlamellar space, which in places is divided by the interlamellar partitions or septae into a series of vertical compartments called the water tubes. The septae end close to the junction of the demibranchs and the visceral mass, so that the upper portion of the interlamellar space forms a continuous channel along the horizontal axis of each demibranch. This condition is found only at the upper (dorsal) part of the body at the level of the stomach and above it (fig. 116, *A*). At approximately the level of the heart the entire gill structure has only three points of attachment, in the center at the visceral mass and at each side where the gills fuse with the mantle (fig. 116, B.).

The entire chamber system of the gills can be visualized as four passages which at the level of the heart merge into two epibranchial chambers. The

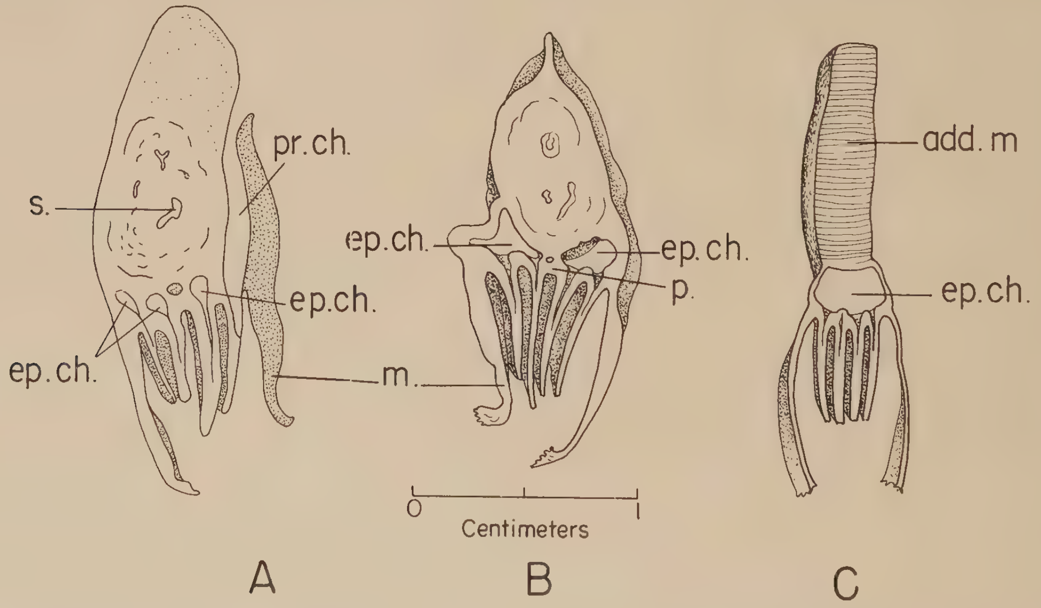

FIGURE 116.—Transverse sections of 1-year-old *C. virginica* (diagram drawn from sectioned and stained preparations). A—Section at the level of the stomach, s.; four epibranchial chambers at the base of the gills, ep.ch., and promyal chamber, pr.ch., at right. B—Section near the level of the heart, two epibranchial chambers, ep.ch., are separated by the pyloric process, p. C—Section at the level of the adductor muscle below the heart, common epibranchial chamber, ep.br.ch. Bouin, hematoxylin-eosin.

latter in turn lead to a common epibranchial chamber which, through the cone-shaped cloaca, opens to the outside.

There are no partitions, valves, or any other features for regulating the flow of water inside the chamber system. The current is maintained solely by the beating of the ciliary epithelium of the gills and of the lining of the chambers. Below the level of the heart the fusion of the gills with the median axis is lost and the two chambers, separated here by the pyloric process of the visceral mass (fig. 72, py.p.), merge together to form a commom epibranchial chamber (figs. 72 and 116, ep.br.ch.). This leads to a wide cone-shaped exhalant chamber or cloaca (fig. 72, cl.) which can be examined by forcing apart the posterior end of the valves and focusing a beam of light on its inner surface. The water tubes appear as large, round holes (fig. 72, w.t.).

PROMYAL CHAMBER

In oysters of the *Ostrea* type all the water collected by the gills is discharged through a single opening of the cloaca. In *Crassostrea*, however, the exhalant system is modified by the presence of an asymmetrical space on the right side of the body called the promyal chamber (fig. 116, pr.ch.). This irregularly shaped pocket between the mantle and the visceral mass extends in a dorsoposterior direction from the level of the pericardium to a wide outside aperture which may be seen by forcing the valves apart and examining the space between them. Openings of the water tubes similar to those found on the inner wall of the cloaca are visible inside the chamber (fig. 75)

The promyal chamber was first observed by Kellogg (1892), who suggested that the water from the gills may be discharged through it. Stafford (1913) showed the chamber in one of the illustrations of his book but did not refer to it in the text. The full anatomical significance of the promyal chamber in *C. virginica* was described by Nelson (1938) and by Elsey (1935) for *C. gigas*.

The position occupied by the promyal chamber makes it apparent that water from the dorsoanterior part of the right demibranch is discharged through this chamber and does not pass through the cloaca. After releasing carmine suspension near the gills of an actively feeding oyster (*C. virginica*), one can observe some of the red discolored water being expelled through the promyal chamber while the principal stream is passing through the cloaca. In spawning males the sperm shed from the right gonoduct is frequently discharged through the promyal chamber.

In assessing the relative importance of the promyal chamber in the movement of water through the gills, Nelson (1938) states that out of 36 water tubes in the right demibranch of *C. virginica* the first 14, comprising more than half the length of the demibranch, are in free communication with the promyal chamber. The remaining 22 water tubes discharge water into the narrowed portion of the epibranchial chamber beneath the adductor muscle or into the cloaca. In the absence of measurements of the amount of water discharged through the promyal chamber, it is impossible to state what percentage of the total output is discharged through the chamber. From anatomical evidence it may be concluded that the greatest part of the water used for ventilation of the gills leaves through the cloaca and only a minor portion through the promyal chamber.

Relative dimensions of the different parts of the exhalant system of the gills can be demonstrated clearly by casts made with plaster of Paris or with latex. Before being injected, the oyster should be completely narcotized. The material used for injection is then forced through both the cloaca and the promyal chamber while the oyster is gently and frequently tapped to permit good penetration to the very ends of the water tubes. When all the passages are full the valves are pressed together and tied with a string. The material is permitted to set for 24 hours before the shells are opened and the soft parts of the oyster removed.

The various parts of the water exhalant system of *C. virginica* are shown in figure 117, which represents the cast of the inner gill chambers viewed from three different angles. The promyal chamber (fig. 117, left, pm. ch.) occupies an irregular area on the right side of the visceral mass and ends in an aperture almost twice as large as the opening of the cloaca (cl.). The relation between the promyal chamber and the two demibranchs of the right side is shown in the central drawing (fig. 117) of the cast viewed from the left side. The right and left demibranchs are separated by a septum (s.) in the upper portion of the posterior side of the gills (fig. 117, right). Most of the inner spaces of the gills are in direct connection with the cloaca; less than one-half of the right demibranch empties into the promyal chamber.

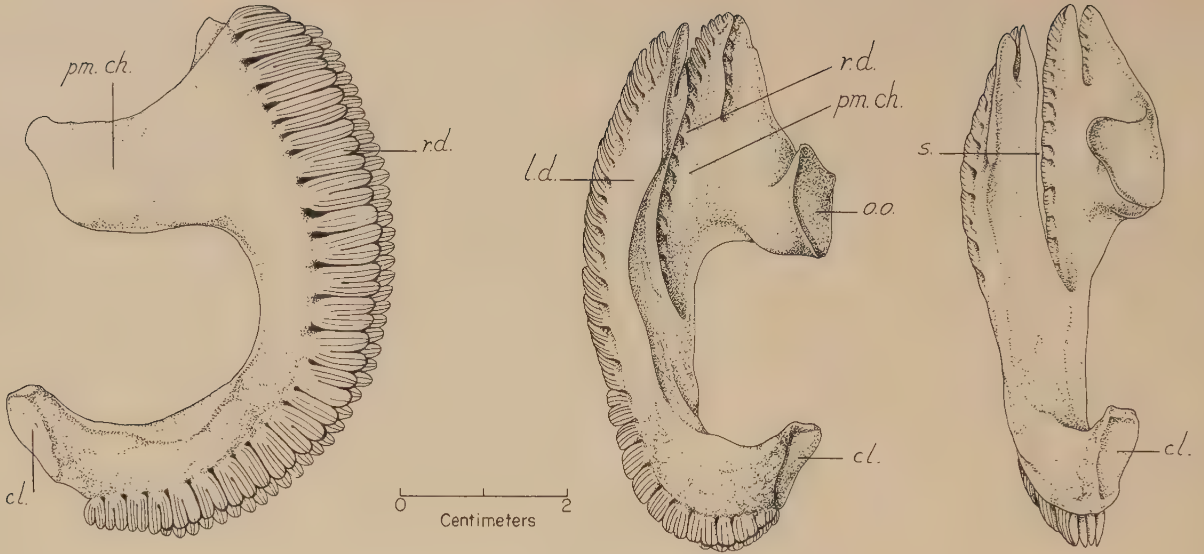

FIGURE 117.—Water exhalant system of the gills of *C. virginica*. Plaster of Paris cast of a large specimen. Left—view from the right side. Center—view at sharp angle from the left. Right—view from the posterior side of the gills. cl.—cloaca; l.d.—left demibranch; o.o.—outside opening (aperture) of the promyal chamber; pm. ch.—promyal chamber; r.d.—right demibranch; s.—septum separating the right and left demibranchs.

The relative size of the promyal chamber is variable. In some specimens it extends over three-fifths of the length of the gills and forms a spacious pocket (fig. 118). The exhalant system of *C. gigas* (fig. 119) is similar to that of *C. virginica*. In the specimens I examined, the promyal chamber extends approximately one-half the length of the gill, and the funnel-shaped cloaca was much wider than in *C. virginica*. In *O. edulis* the promyal chamber is absent and the entire system of water tubes, epibranchial chambers, and cloaca is symmetrical (fig. 120). In comparison with *C. virginica* and *C. gigas*, the cloaca of *O. edulis* is much broader and longer, which is probably the result of the almost circular shape of the body. The water tubes are shorter than in *Crassostrea*.

The promyal chamber also has been described for the Australian rock oyster, *Ostrea* (*Crassostrea*) *cucullata*, Born (*C. comercialis* I. and R.), and in *Ostrea* (*Crassostrea*) *frons* L. from the mangrove roots in Florida (Nelson, 1938).

Oysters of the *Crassostrea* type inhabit and thrive in the brackish and muddy coastal waters, which are less suitable for the mollusks of the *Ostrea* type. It has been implied by some investigators (Elsey, 1935; Nelson, 1938) that the tolerance to muddy waters is due to a superior cleaning mechanism which is somehow associated with the presence of the promyal chamber and prevents

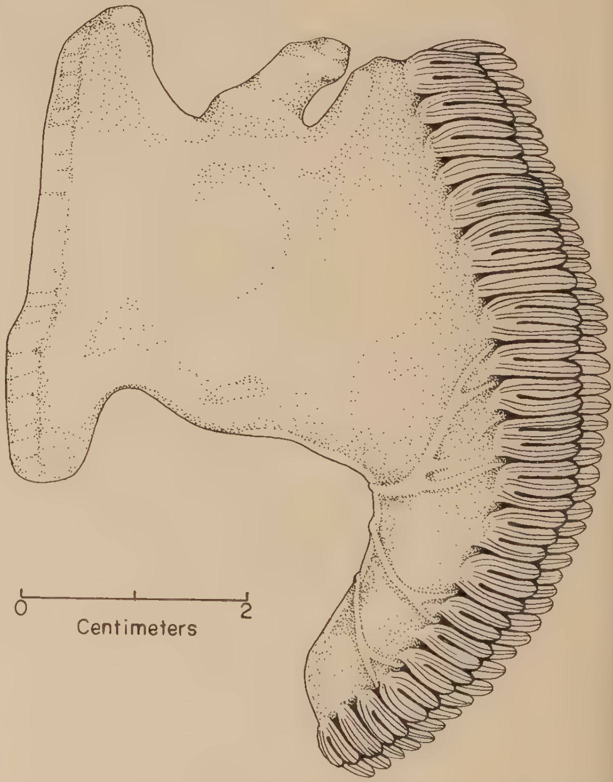

FIGURE 118.—Cast of the water exhalant system of a very large *C. virginica*. Note the extent of the promyal chamber along the gill axis and the impression made by the distended pallial arteries at the lower end of the right demibranch.

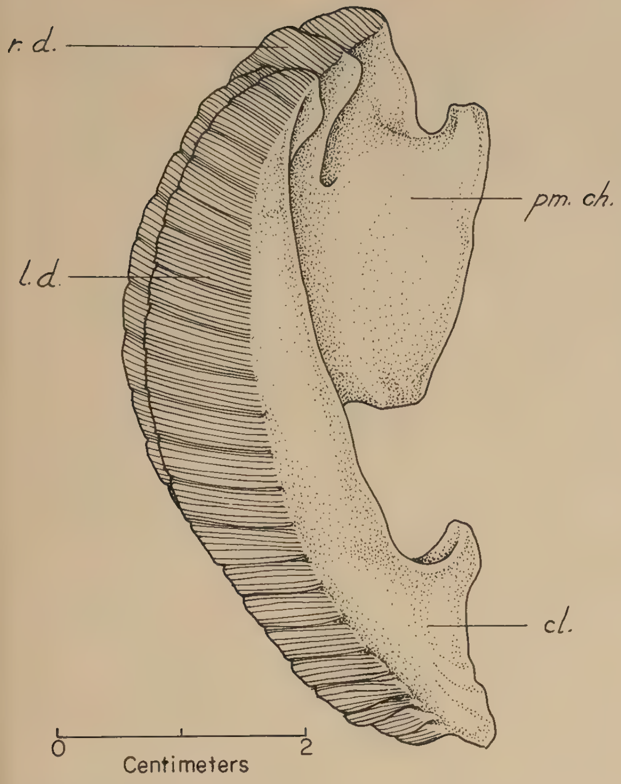

Figure 119.—Cast of the water exhalant system of the gill of *C. gigas* viewed from the left side. cl.—cloaca; l.d.—left demibranch; pm. ch.—promyal chamber; r.d.—right demibranch.

the accumulation of sediment in the pallial cavity. No evidence has yet been presented to corroborate this view. The physiological significance of the chamber is not known beyond the fact that it provides an additional outlet for the discharge of water through the gills. Elsey (1935) remarks that in *C. gigas* the promyal chamber allows a free passage of water when the gonads distend before spawning and encroach into the branchial space, partially closing the water tubes. Sections of the visceral mass of *C. virginica* with fully developed gonads do not show any "encroachments" into branchial space. The latter remains unobstructed, and the water tubes fully open. At present it is difficult to see what structural and physiological advantages are gained by the presence of a promyal chamber in *Crassostrea* oysters.

GILL LAMELLA

Each lamella of a gill consists of a great number of tubular filaments arranged at right angles to the axis of the gill. At the edge of the plate the filaments are reflected on themselves and continue upward along the plane of the ascending lamella. The filaments forming the gill-lamella do not lie smoothly on one plane; they are arranged in a series of transverse folds or plicae that give the surface of the gill a plaited appearance noticeable to the naked-eye. A transverse section (fig. 121) shows the arrangement of filaments in alternating grooves and ridges. The number of filaments on a single fold of an adult *C. virginica* is not constant. In my preparations it varied from 10 to 16 per fold.

There are three types of filaments that can be distinguished by their position, shape, and dimensions. The larger, or principal filaments (fig. 121, p.f.), are located at the bottom of the groove between the plicae. In cross section they have a triangular shape with two bulky chitinous rods forming two sides of this triangle. The rods are fused at the apex but are separated at the base, which contains a narrow blood vessel. The two transitional filaments (t.f.), one on each side of the principal one, are smaller and differ in shape from the ordinary filaments (o.f.), which form the rest of the plica. Sometimes the difference is insignificant. In general the ordinary filaments seen on cross section are elongated, club-shaped units.

Throughout their length the filaments of each plica are joined at the bases by regularly spaced interfilamentar junctions (if.j.), which consist of narrow bands of vascular connective tissue. The free portions of the filaments surround the oval-shaped openings, called ostia or fenestrae (o.), through which the water enters the inside passages of the gill. Numerous muscle fibers follow the interfilamentar junction and extend along both sides of the plica to its distal part (if.m.). At intervals, the two lamellae of each demibranch are connected by the partitions (interlamellar septa) made of connective tissue (il.s.) which run across the plate from one lamella to the other. These partitions are more numerous toward the distal (free) edge of the demibranch and diminish toward the base of the gill, where they are found only at about every sixth plica. The interlamellar junctions contain numerous muscle fibers, blood vessels, and nerves. The muscle fibers are arranged in three systems: the longitudinal muscles (l.m.) seen in cross section extend vertically from the proximal to the distal end of the lamellae; the transverse muscles (tr.m.) go from one side of the lamellae to the other; and the tangential

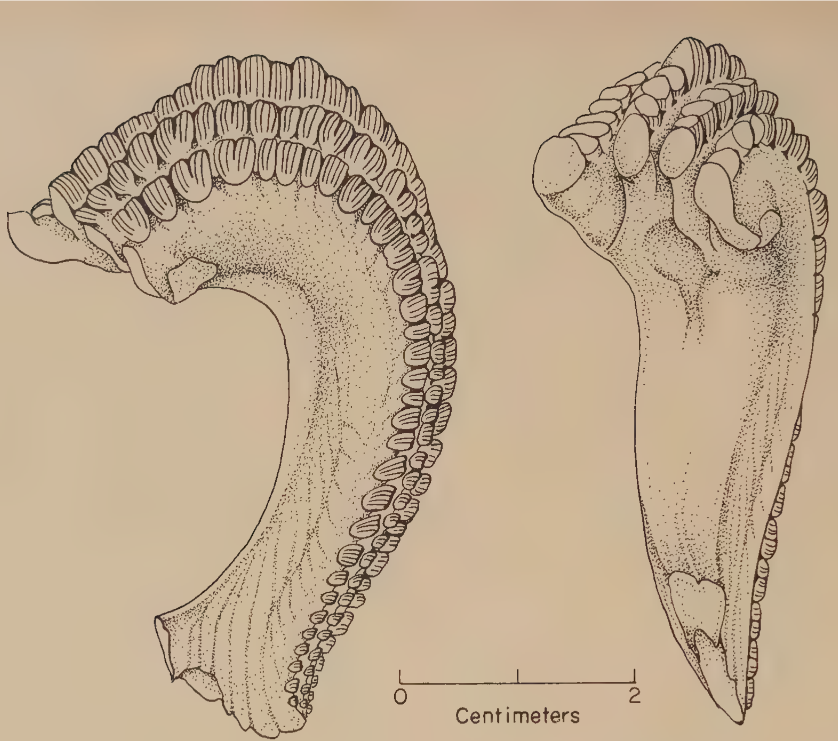

FIGURE 120.—Inner cast of the water exhalant system of the gills of *O. edulis*. Right—viewed from posterior side; Left—viewed from left side.

interlamellar muscles (il.m.) underlie the surface epithelium of the junction between the ascending and descending lamellae. At both ends of the junction near the location of the principal filaments the muscles branch off and form well-pronounced bands underlying the chitinous rods. It can be deduced from the pattern of distribution of the interlamellar muscles that their contractions bring the plicae of the two sides of the demibranch together, constrict the blood vessels, and reduce the diameter of the water tubes.

SKELETON

A framework of chitinous rods forms a scaffolding which supports the entire gill structure. The skeleton can be isolated from the tissues by brief treatment with a weak solution of sodium hydroxide, which does not affect the chitin. Structural elements cleared by this method are shown in figure 122. The skeleton of each filament resembles a ladder with the horizontal rungs slightly curved on one side and joined to the vertical elements by knobs of chitin. On both sides the supporting ladderlike unit of each filament is joined by cross pieces to the next units, forming a continuous framework. The vertical and horizontal bars surround the openings (ostia) between the filaments (o.). Each skeleton unit supports two adjacent filaments. The vertical bars correspond to the walls of the two adjacent filaments, while the horizontal members (the rungs of the ladder) are embedded in the tissue of the interfilamentar junctions. Two purposes are accomplished by such a pattern. The gill acquires rigidity and at the same time provides strong support for the delicate, sievelike membrane through which water passes into the water tubes. Heavy rods support the principal filaments (fig. 123). At the base of the gills the skeleton rods form massive V-shaped arches embedded in fibrous connective tissue (fig. 124).

THE FILAMENTS

The structural unit of the gill is a tubular filament of ciliated epithelium supported by skeleton rods. The central part of the filament is occupied by a space which is periodically filled with blood as the gill plates expand and contract. Connective tissue underlies the proximal part of the filament which consists of nonciliated, almost cuboid cells, tightly packed in a two-cell layer. The distal part of the filament is covered with ciliated cells (fig. 125). Bulky mucous cells occur at irregular intervals and discharge their

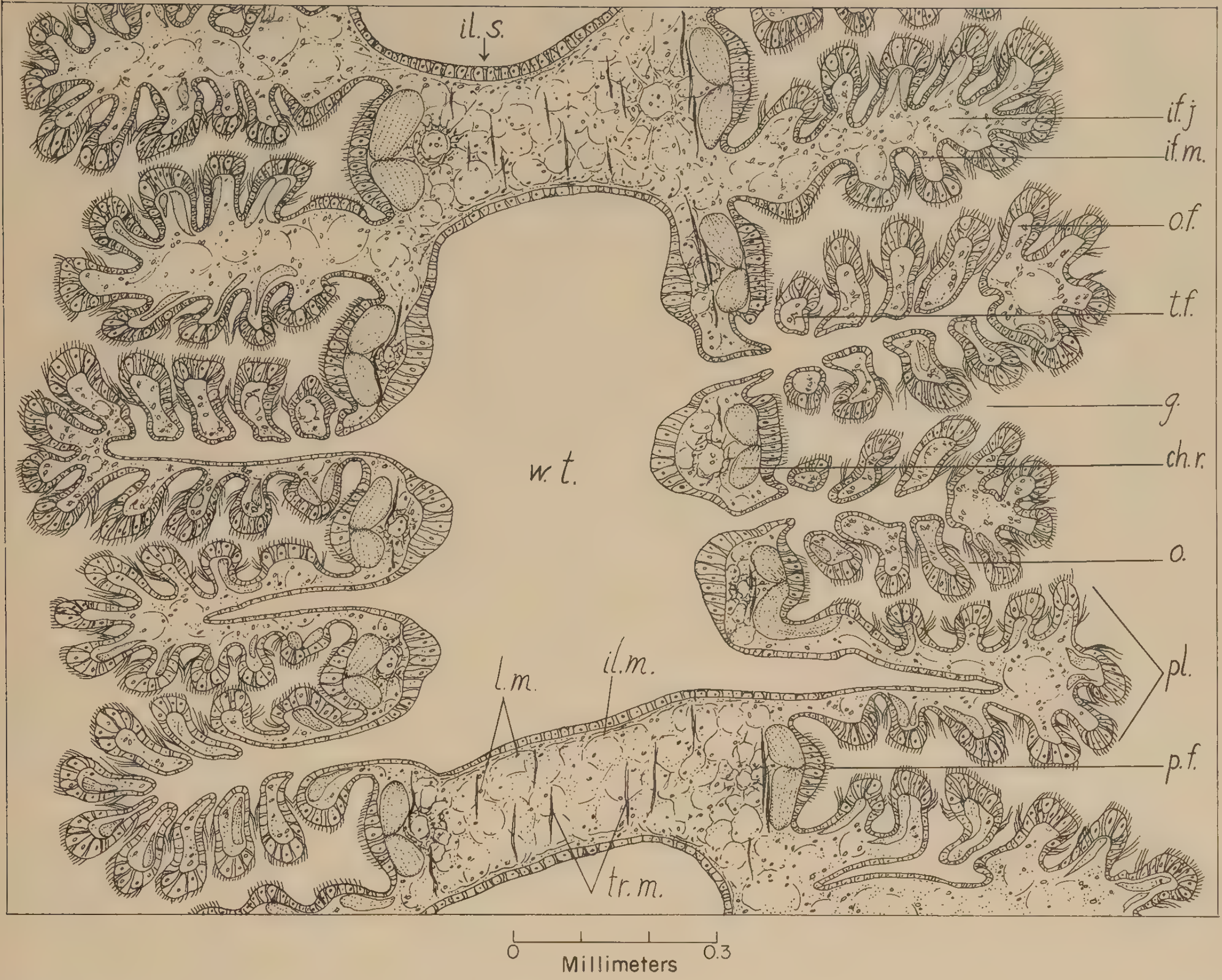

FIGURE 121.—Transverse section of the demibranch of *C. viriginica*. Kahle fixation, hematoxylin-eosin. ch.r.—chitinous rods; g.—groove; if.j.—interfilamentar junction; il.m.—interlamellar muscles; il.s.—interlamellar septum; l.m.—longitudinal muscles of the interlamellar septum; o.—ostium; o.f.—ordinary filament; p.f.—principal filament; pl.—plica; t.f.—transitional filament; tr.m.—transverse muscle of the interlamellar septum; w.t.—water tube.

content to the surface of the gills. The secreted mucus spreads over the gill plates and entangles the particles which settle on them. At the distal edge of the gill the filaments are fused together to form a terminal groove along which food is conveyed toward the mouth. The principal filaments differ from the others by their larger size and nontubular appearance. At the base of each filament there is a blood vessel located in a space between the chitinous rods. The epithelial cells are slightly larger than those of the ordinary filaments and have longer cilia (fig. 126).

OSTIA

Ostia or fenestrae, the oval-shaped open spaces between two adjoining filaments (figs. 121 and 125, o.), are framed by two vertical and two horizontal skeleton bars covered with epithelium. Their configurations and dimensions vary, depending on the degree of contraction of the filamental musculature and the distention of blood spaces. In an actively feeding oyster the contraction and expansion of the ostia regulate the passage of water through the gills. This can be observed on the surface of a gill exposed by cutting off a piece of shell. The dimensions of the ostia are somewhat correlated to the size of the oyster ova which pass through the gills during spawning (see ch. XIV, p. 303). In a viviparous *O. lurida* the ostia are large, varying from 90 by 45 μ in contracted state, to 180 by 60 μ when fully expanded. The ova of this species average 90 μ in diameter. In *C. gigas* and *C. virginica*, which have smaller eggs,

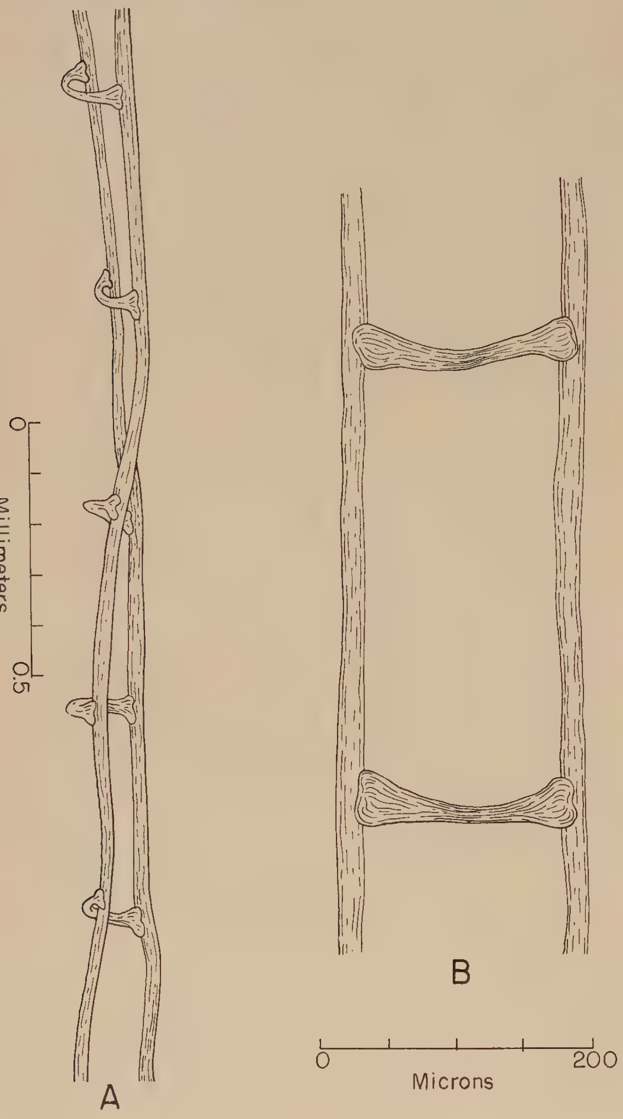

FIGURE 122.—Side view (A) and front view (B) of a skeleton of ordinary gill filaments of *C. virginica*. Soft tissues removed by sodium hydroxide. Unstained preparation, whole mount.

the ostia are approximately only one-third the dimensions of those in *O. lurida*.

CILIARY TRACTS

The surface of the filament is covered by several different ciliary tracts. Cells of uniform size on the outer surface bear the frontal cilia, which are relatively small and beat parallel to the surface of the filament (fig. 125, fr.c.). They are flanked on each side by a single laterofrontal cell (lf.c.) of larger size with a blade-shaped cilium, which according to Atkins (1938) occurs in the family Ostreidae and is somewhat different from the laterofrontal cells of other bivalves. In fixed and stained preparations this wide and curved cilium is frequently frayed. The shape of the cilium and the presence of the basal granules, typical for normal ciliated cells, both indicate that the laterofrontal cilium is formed by the fusion of ordinary filaments, a view which is confirmed by studies made with the electron microscope (see p. 132). The laterofrontal cells of *C. virginica* are fairly large and cone-shaped; their relatively small nuclei are located at the narrow, proximal end of the cell; and the protoplasm is devoid of granules and deeply stained with hematoxylin. The laterofrontal cilia of gills preserved in a relaxed state extend toward their opposing numbers on the adjacent filament and touch their tips. In a contracted gill they are bent and almost undistinguishable from the cila of the frontal cells. The length of the laterofrontal cila on sectioned and stained preparation varies from 11 to 15 μ. Accurate measurements, however, are difficult because of the bending of the cilia which do not remain fully extended even in completely narcotized cells. In *O. edulis*, according to Atkins (1938), the length of the laterofrontal cilia varies from 14 to 25 μ. The

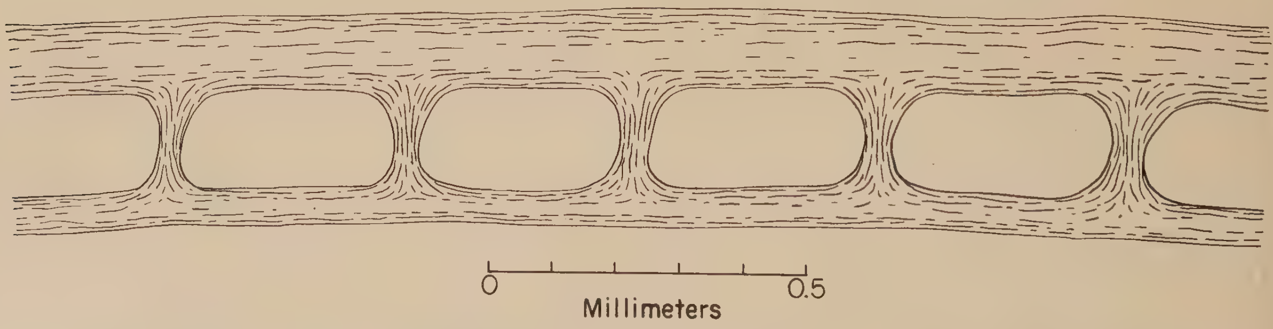

FIGURE 123.—Chitinous rod of the principal filament of *C. virginica*. Soft tissues removed by sodium hydroxide. Unstained preparation, whole mount.

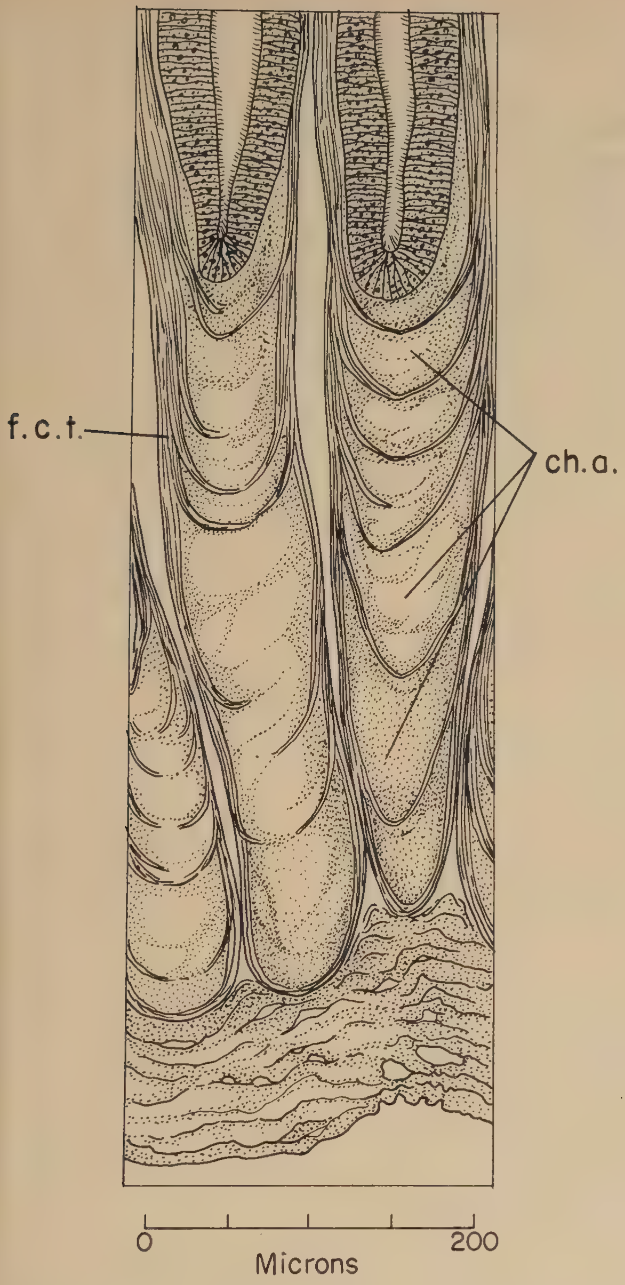

FIGURE 124.—Longitudinal section near the base of the gill of *C. virginica*. Chitinous arches, ch.a., are embedded in fibrous connective tissue, f.c.t. Osmic acid fixation, iron hematoxylin.

frontal and laterofrontal cilia of the principal filaments have the same structure as those of the ordinary filaments, differing only in their greater size. A short distance below the cell surface each cilium terminates in a basal body, a tiny granule from which a pair of rootlets extends deeper into the protoplasm and becomes undistinguishable near the nucleus (figs. 126 and 127).

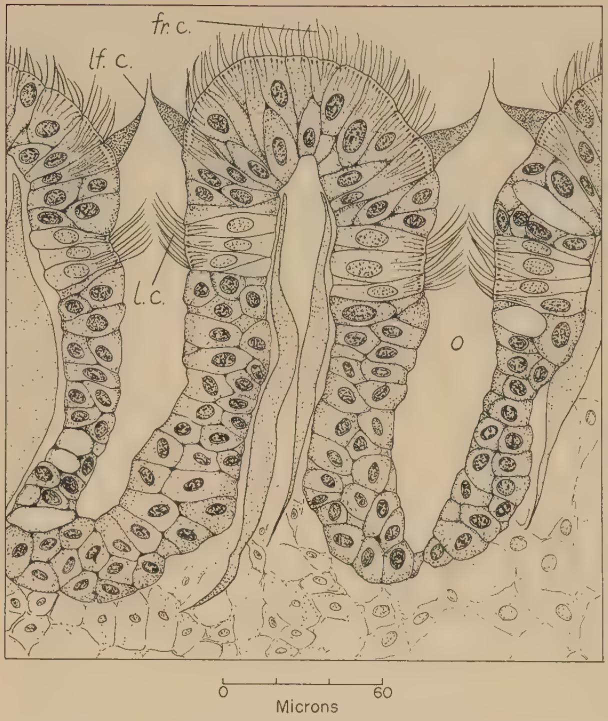

FIGURE 125.— Transverse section through ordinary filament of *C. virginica*. Vertical chitinous rods (stippled areas) and blood space are at the center. fr.c.—frontal cilia; lf.c.—laterofrontal cilia; l.c.—lateral cilia; o.—ostium. Kahle fixation; hematoxylin-eosin.

Besides the frontal and laterofrontal cilia, Atkins (1938) distinguishes in *O. edulis* the "fine frontal" and "paralaterofrontal" cilia, which run on both sides of the central portion of the frontal tract (fig. 127, f.f.c., para l.f.c.). He states (1938, p. 367) that: "Subsidiary laterofrontal cilia are present in Ostreidae, but are very difficult to distinguish even in the living gill." I was unable to identify these cells in sectioned preparations or in the living gill filaments of *C. virginica* examined under high power. The frontal cilia of this species appeared to be of uniform length along the entire cross section of the tract (fig. 125).

Beneath the laterofrontal cilia of the filaments there is a group of six large cells, four of them broad and two narrow, which bear large, stout cilia about 17 to 18 μ in length. These are the lateral cilia (fig. 125 and 127, l.c.), which bend forward slightly toward the outer surface of the filament and touch the cilia of the opposite group.

TERMINAL GROOVE

The free edge of a demibranch formed by the concrescence of the ascending and descending lamellae is a shallow trough called the terminal

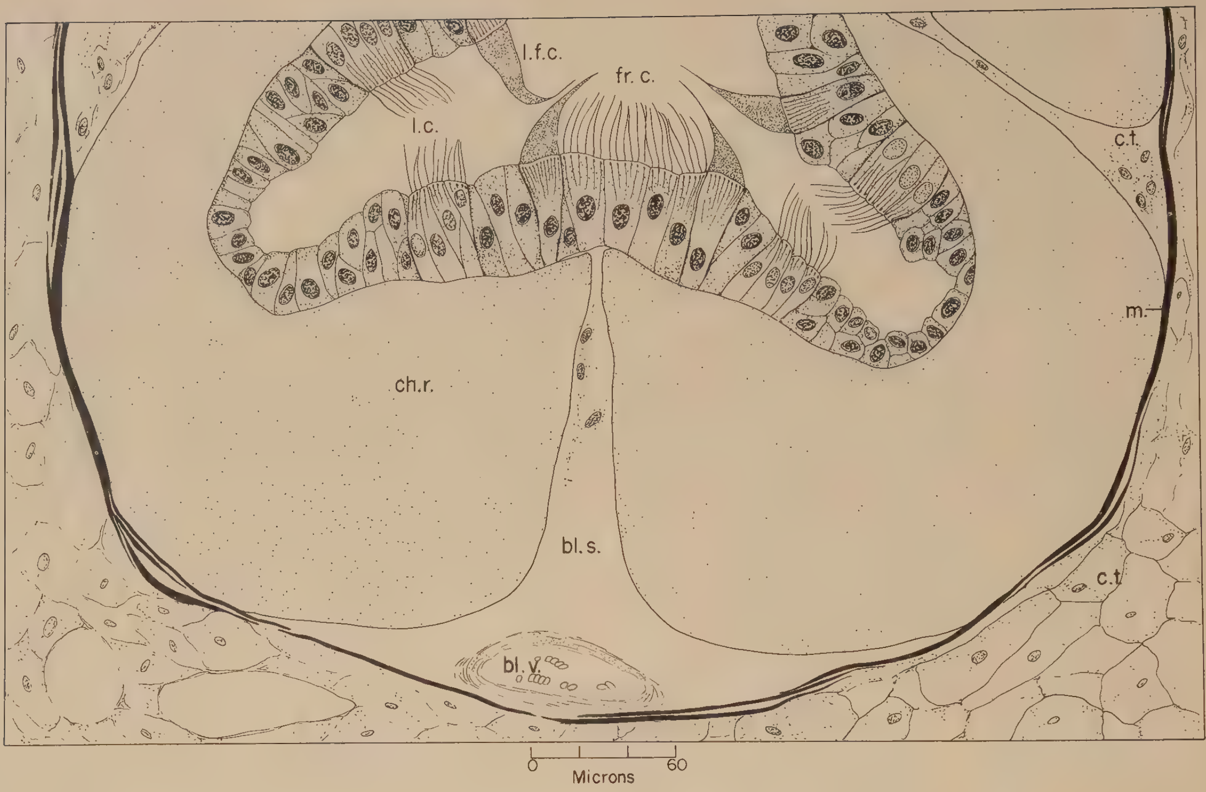

FIGURE 126.—Transverse section of the principal filament of the gill of *C. virginica* drawn from the same preparation as in figure 125. Note the well-developed muscle fibers, m., under the large skeleton bars, ch.r.. c.t.—connective tissue; bl.v.—blood vessel; bl.s.—blood space; fr.c.—frontal cilia; lf.c.—laterofrontal cilia; l.c.—lateral cilia; m.—muscle.

groove. This depression at the border of the gills extends their entire length. The epithelial lining of the terminal groove consists of columnar ciliated cells with large cilia and numerous mucous and eosinophilic cells. The epithelium rests on a basal membrane. Transverse muscle fibers extend between the two sides of the groove. During feeding the grooves are open, the condition which is shown in figure 128. Their contraction brings the edges together and closes the groove. In this way the oyster discards some of the material which was collected by the surface of the gill. The rejected particles entangled in mucus are dropped to the inner surface of the mantle and are discharged. The direction of the ciliary beat along the four terminal grooves is always toward the labial palps and the mouth.

THE MUSCLES OF THE GILLS

The gills of an actively feeding oyster contract and expand at frequent, although irregular, intervals. This behavior is difficult to notice in an intact oyster, but it can be observed in an oyster in which much of the valve has been cut off without injuring the adductor muscle and the gills. The mantle at the exposed area rolls up and leaves the gills in full view, and if carefully performed, the operation has no visible ill effect on the function of the gill.

The most conspicous movements which can be seen with the naked eye are the muscular contractions at the bases of the gills and the corresponding changes in the position occupied by the demibranchs. These four structures may stand apart like stiff leaves of a wide open album or they remain parallel, touching one another like the pages of a closed book. There is also a lateral movement of the filaments which brings them together or pushes them apart. This movement frequently occurs independently of the contractions of the demibranchs and may be limited to a small portion of the plica. Both types of movements affect the opening of the ostia, which are widely stretched when either the four demi-

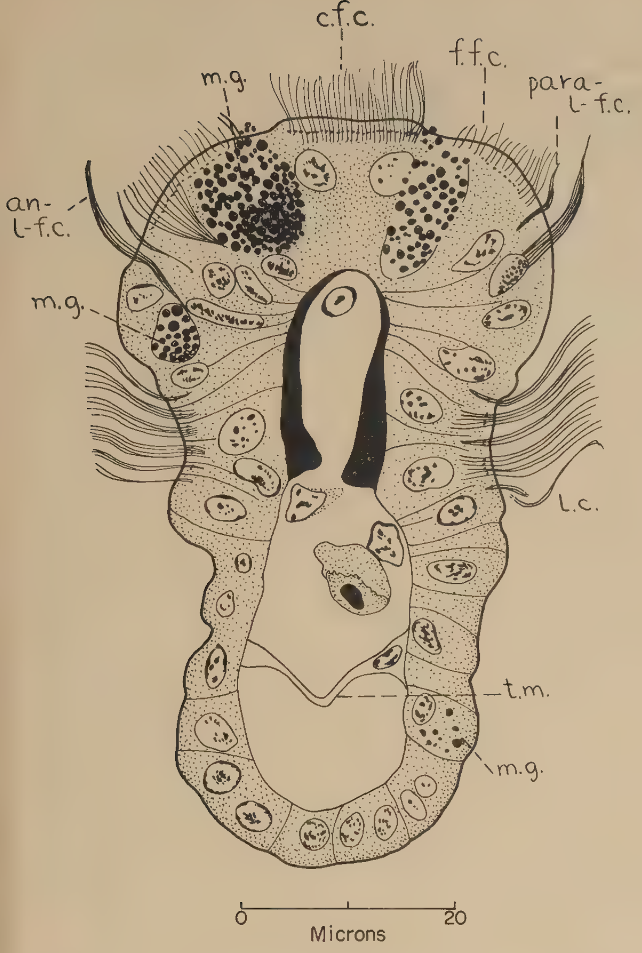

Figure 127.—Cross section of the filament of the gill of *O. edulis*, according to Atkins, 1938. an.l.fc.—anterior laterofrontal cilia; c.f.c.—central frontal cilia; f.f.c.—fine frontal cilia; para l.fc.—paralaterofrontal cilia; m.g.—mucous gland; l.—lateral cilia; t.m.—transverse muscle fiber. Chitinous rods are shown as black areas under the epithelium.

branchs or only a group of filaments stand apart and are constricted when the latter are drawn together.

Changes in the position of the demibranchs depend on two distinct systems of muscles located at the gill axis above and below the skeletal arches. In general the muscle fibers follow the configuration of the arches. The larger bands located inside the arches are the flexor muscles, which are attached to the inner sides of the two arms of an arch (fig. 129, f.). Their contraction brings the two adjacent demibranchs together. The smaller bands at the base of the arch (ex.) are the extensor muscles, which cause the demibranchs to stand apart. The action of the two bands shown in the figure is antagonistic. The extensor bands are smaller, probably because the elasticity of the chitinous arches pushes the demibranchs apart and this springlike action means that less force is required of the extensor muscles than of the flexor bands.

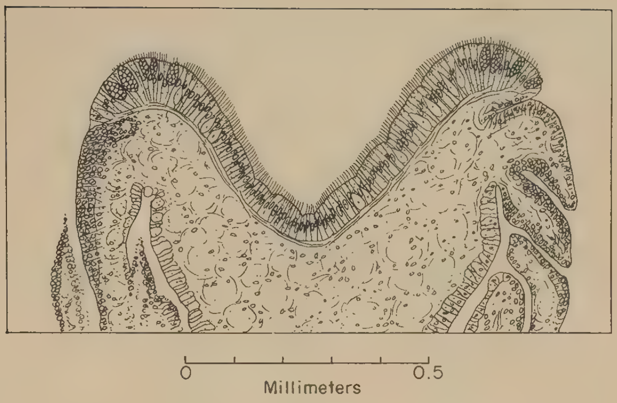

Figure 128.—Terminal groove at the edge of a demibranch of *C. virginica*. Longitudinal section of the demibranch. Bouin, hematoxylin-eosin.

Other muscle bands of the gills, although less conspicuous than the flexors and extensors of the arches are, nevertheless, of great importance in regulating the transport of water through the complex gill apparatus and in facilitating the exchange of blood inside the gill filament. Water tubes of the gill can be constricted by the contraction of the muscles underlying the epithelium of the interlamellar septa and extending from one lamella to another (fig. 121, il.m.), while the contraction of the transverse muscles of the interlamellar septa compresses the blood vessels. The contraction of the longitudinal muscles of the septa (fig. 121, l.m.) results in the withdrawal and shortening of the entire demibranch. This reaction occurs spontaneously but can also be induced by stimulation. The contraction of the interfilamental muscles (if.m.) brings together the vertical rods of the gill skeleton, causes the curving of the crossbars, and constricts the blood space of the filament, forcing blood into the pallial veins.

Contractions affecting only part of the gill cause the blood to oscillate inside the gills. Because of the open nature of the lamellibranch circulatory system the direct return of blood from the gills to the auricles cannot be accomplished by the pumping action of the heart. Contractions involving the entire gill apparatus are needed to complete the renewal of blood.

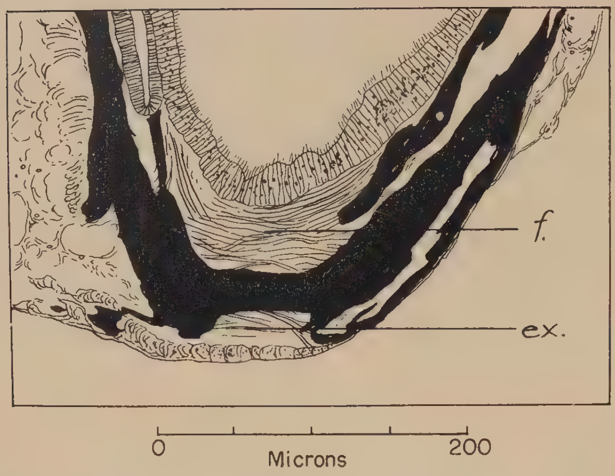

Figure 129.—Longitudinal section through the base of a demibranch of *C. virginica*. Kahle, Mallory triple stain. ex.—extensor muscles; f.—flexor muscles. Pieces of the skeleton arch are shown in black.

CILIATED CELLS

The structure and function of vibratile elements of the cells have been the object of numerous investigations beyond the scope of this book. The reader is, therefore, referred to comprehensive reviews of the problem of ciliary motion made by Gray (1928) and more recently by Atkins (1938) and Brown (1950). Several theories based on studies of the structure and action of cilia fail to give a satisfactory explanation of ciliary motion, which at present still remains a biological mystery.

Cilia examined in transmitted light or viewed on a dark background in reflected light appear to be optically homogenous. In polarized light they are birefringent (Schmidt, 1937). Observations with the light microscope disclose the presence of an axial filament (axoneme) surrounded by a thin sheet of cytoplasm (Wenyon, 1926). As a rule, the cilia emerge from tiny basal granules near the cell surface and penetrate through the cuticle, which under the light microscope appears as a thin homogenous membrane. Studies of the role and origin of basal bodies in various ciliated cells have resulted in a great number of speculations. Experiments by Peter (1899) showed that in small fragments of a crushed protozoan the cilia continued to beat as long as they were in organic connection with the adjacent pieces of cytoplasm. He deduced from this observation that the ciliary mechanism is located near the surface of the cell. Similar results were obtained with lateral cells stripped away from the filaments of *Mytilus* gills. The cilia that were removed from the basal granules remained motionless while those connected with them continued to beat (Gray, 1928). The microdissection technique in more recent years supports these findings. It was demonstrated that in the ciliated cells of the gills of *Anodonta* the motion of the cilia ceases when the cell is cut transversely in the immediate region of the basal granules. Transverse cuts made at any level within the proximal two-thirds of the cell had no effect on ciliary motion, but if the cut was made across the zone occupied by the fibrillae or rootlets in the distal third of the cell, the coordination of the ciliary motion was destroyed although the cilia continued to beat. These observations seem to support the validity of the theory, advanced independently by Henneguy (1897) and Lenhossék (1898), that the basal granule, homologous and sometimes identical with the centrosome of the mitotic figure, is the center which controls the activity of the cilium.

FINE STRUCTURE OF THE CILIA

With the advance of electron microscopy considerable progress has been made in the study of the fine structure of cilia. It has been discovered that throughout the plant and animal kingdoms, regardless of the position of the organism on the evolutionary level and irrespective of the organs studied, cilia have a common structural pattern. The cilia of the gill epithelium of the oyster are no exception to this rule. Thin sections of the frontal and lateral cells of the filaments fixed in buffered osmic acid and examined under the electron microscope show a structure which is undistinguishable from that of the cilia of vertebrates, protozoa, or the tails of spermatozoa. The cilium consists of a protoplasmic matrix in which are embedded 11 filaments; 2 single filaments are at the center and 9 double ones are arranged in a ring on the periphery. The central pair is connected to the peripheral ring by radial trabeculae or spokes. Short pieces of dense material join the outer filaments to the membrane (fig. 130), which binds more osmium and is, therefore, darker than their interior, making the cilia appear tubular (Fawcett, 1958). The two central filaments are oval shaped in cross section. The plane in which these filaments are oriented is similar for all the cilia of the cell and is thought to be perpendicular to the direction of the ciliary beat (Fawcett, 1958).

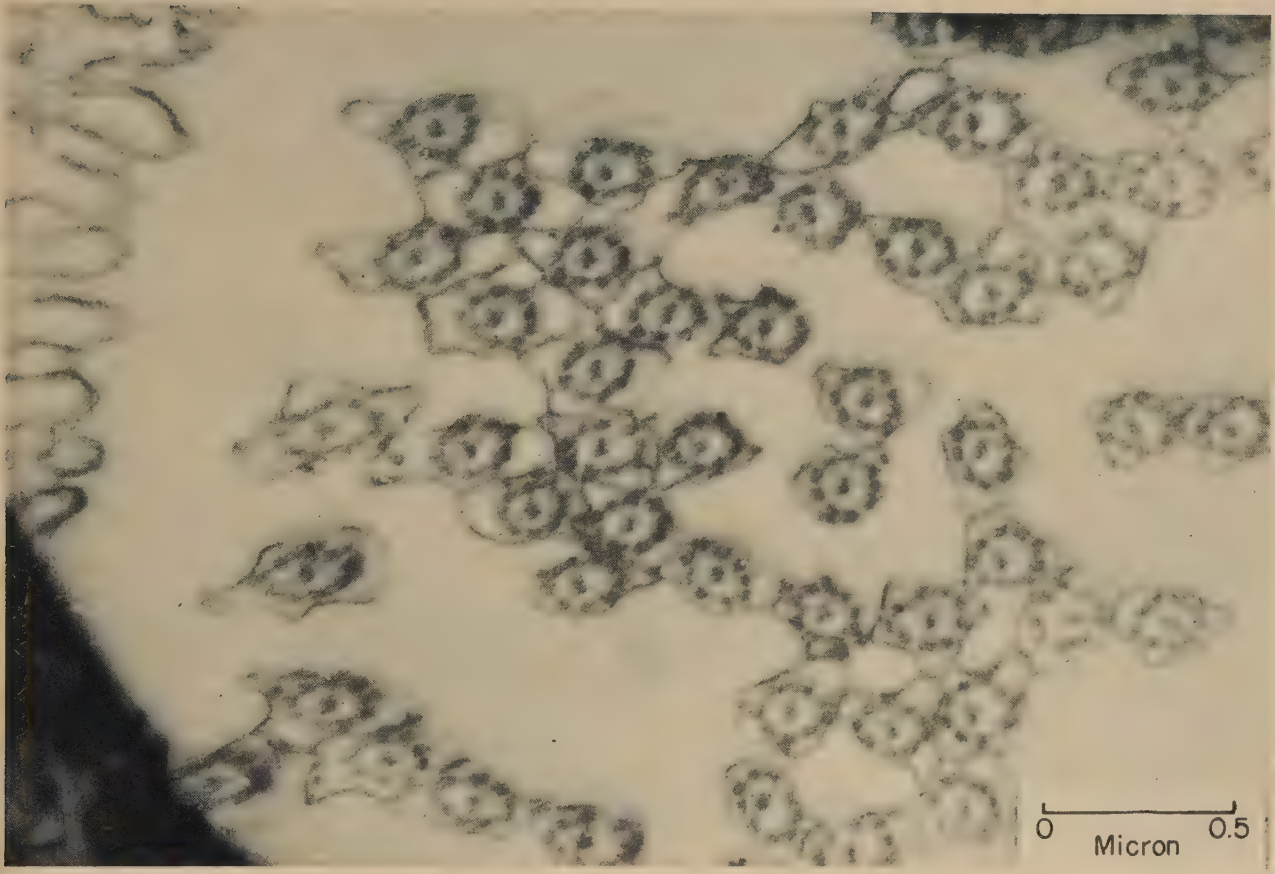

FIGURE 130.—Cross section of the group of frontal cilia of the gill of *C. virginica*. Microvilli of the cell surface are seen at the bottom. Electron micrograph. Buffered osmic acid 1 percent.

The orientation is apparent in the electron micrograph (fig. 131) of a longitudinal section of the distal part of the lateral cell of the filament of *C. virginica* and on transverse sections of the frontal cilia (fig. 130). Because the latter cilia are curved in the direction of the beat, they were cut transversely and appear in the micrograph a short distance above the cell surface. Their oval-shaped axial filaments are oriented parallel to the surface of the cell, i.e., in the direction of ciliary beat. The membranelike laterofrontal cilia consist of several individual cilia embedded in a protoplasmic membrane, but each element retains the typical structure of a single cilium (fig. 131).

The basal corpuscles of cilia are arranged in rows (fig. 132), and the central part of each is surrounded by denser cortex, giving the appearance of an empty central cavity. In the longitudinal section (figs. 131 and 132) they are elongated with a pair of rootlets arising from each proximal end. Rootlets of the cilia of the clam, *Elliptio complanatus*, have a periodic striation of about 750 Å. Similar periodicity appears in electron micrographs of oyster cilia made in the course of my studies, but the picture is not as clear as that published by Porter and Fawcett (see DeRobertis, Nowinski, and Saez, 1954, p. 382).

The distribution of rootlets follows a precise pattern. Each rootlet of a pair turns at an acute angle and crosses over the rootlet of the adjacent corpuscle. The rootlets may be followed further down the cytoplasm toward the nucleus (not shown in the micrograph); some of them cross the second rootlet emerging from the other side of the same corpuscle as can be seen at the center and left side of figure 132. The crossed rootlets are in close contact with each other, but it is not clear whether or not they are fused. Apparently direct communication between the basal corpuscles is lacking.

The question of whether the rootlets are simply the anchoring structures of the cilia or play an active part in its movement remains unanswered.

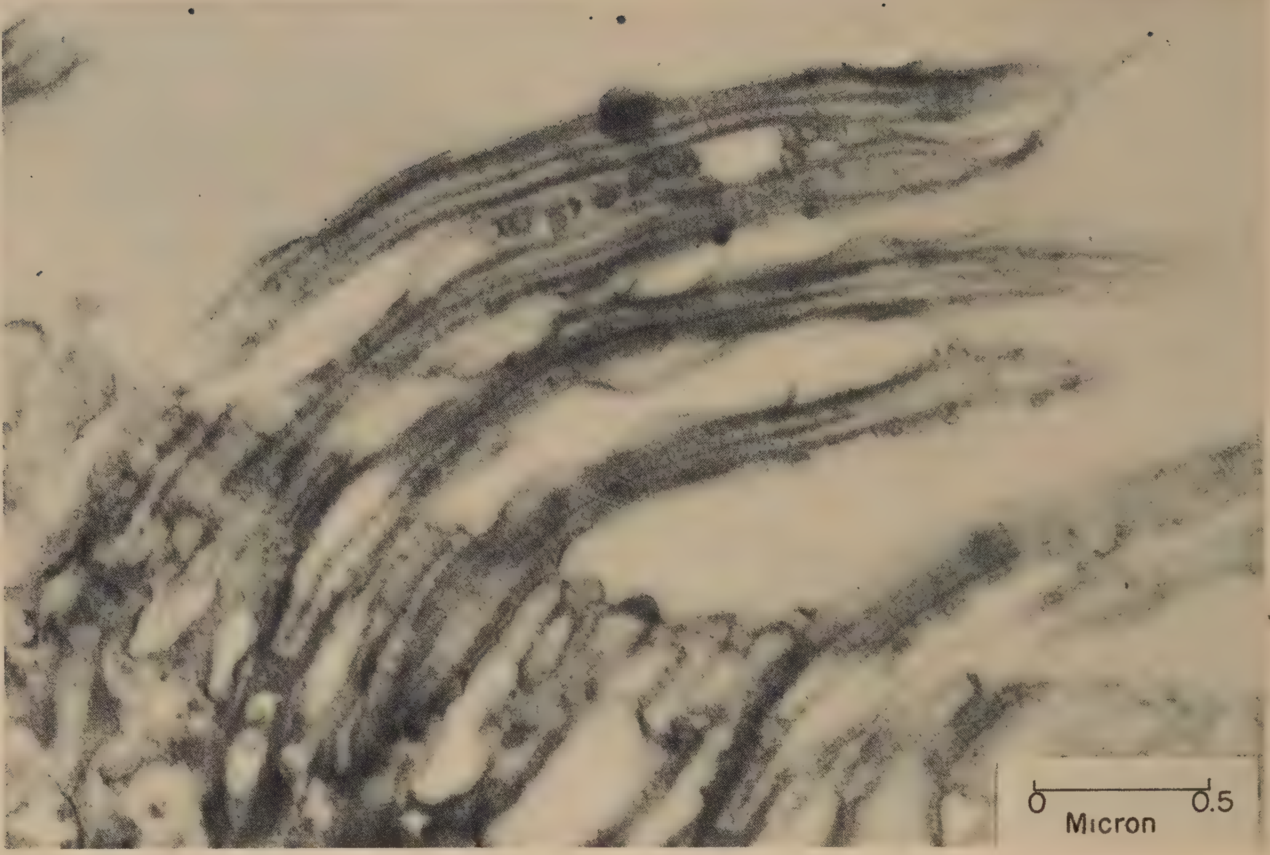

FIGURE 131.—Longitudinal section of the distal portion of laterofrontal cell of the gill of *C. virginica.* Since the plane of section passes at the middle of the cilium only single axial and two peripheral filaments can be seen. The basal corpuscle and the beginning of rootlets are at the lower part of the micrograph. Electron micrograph. Buffered osmic acid 1 percent.

There is the possibility that they may represent a coordinating mechanism of the ciliary epithelium. The fact that the rootlets of the two adjacent corpuscles cross each other is in favor of this view, which was advanced by Grave and Schmitt (1925) on the basis of their observation of the cilia of fresh-water mussels made with the light microscope. Exploration with the electron microscope gives additional support to their hypothesis which, however, requires further corroboration.

The free surface of the ciliated cell appears as a thin homogenous layer, devoid of visible structure, when examined in the light microscope. In reality this layer consists of fingerlike processes called microvilli (figs. 130 and 132), which are found in various tissues; they are considered a device to increase the surface of the cell. Their number has been estimated as high as 3,000 per single cell of intestinal mucosa, and there is no doubt that numerous fingerlike processes greatly increase the surface area of the gill and facilitate the exchange of gases and ions. In figure 132 the layer of microvilli, about 0.5 μ in thickness, rests upon the plasma membrane of the cells. The cytoplasm under the membrane contains numerous mitochondria.

The complex ultrastructure of the ciliated cell of the oyster gill is shown diagrammatically in figure 133, which represents a reconstruction of the principal features seen on electron micrographs. The diagram is based on a large number of micrographs and summarizes our present knowledge of the dimensions and arrangement of the various parts which comprise the ciliated apparatus of the oyster gill.

Although the mechanism of ciliary motion is not known, studies of the ultrastructure of the cilia suggest that the molecular organization of both cilia and myofibrillae of the muscle cells are homologous and that the mechanism of their contraction is similar. This conclusion gains further support from biochemical studies which

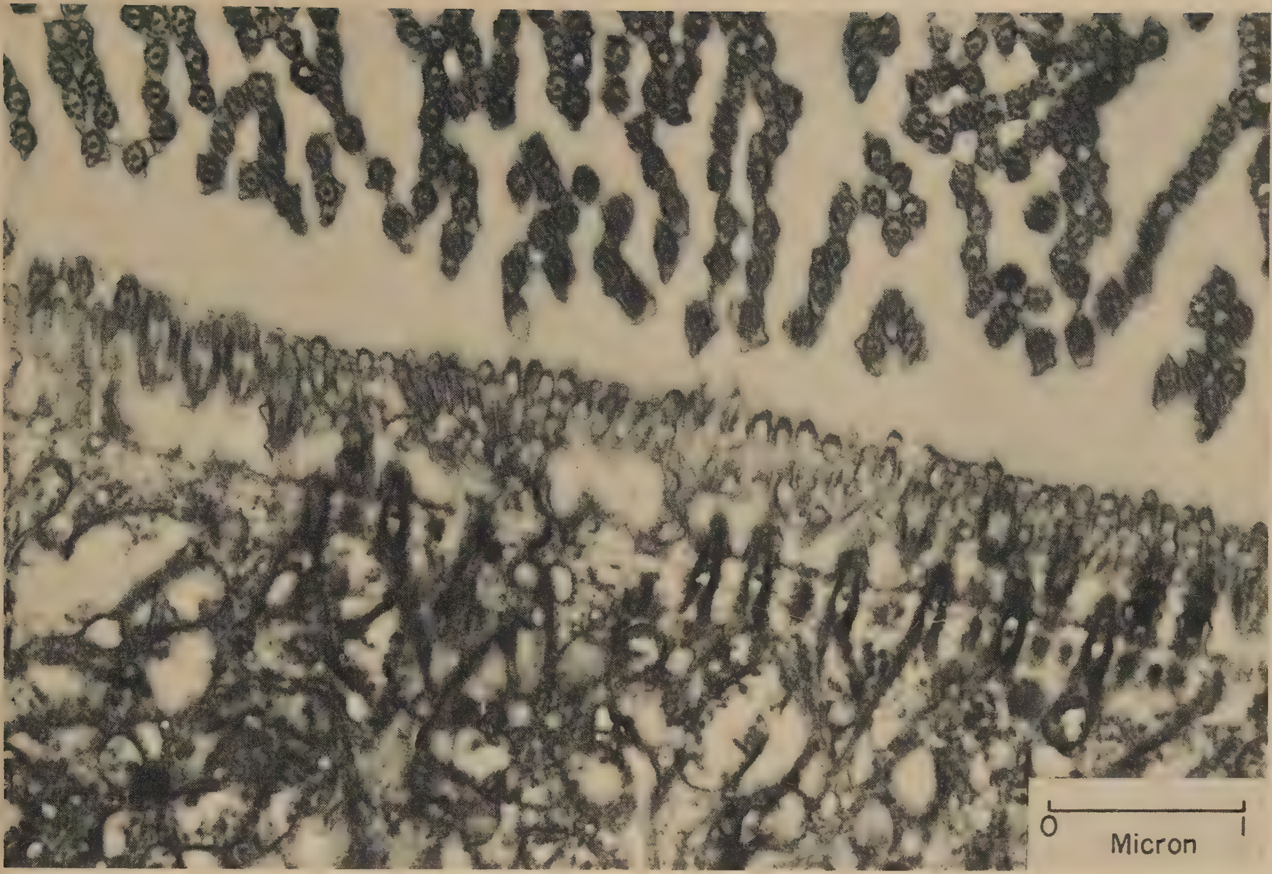

Figure 132.—Section perpendicular to the surface of frontal cilia of the filament of the gills of *C. virginica*. The curved frontal cilia are cross sectioned. Note the row of basal corpuscles with rootlets; the sharp line, parallel to the surface of the cell corresponding to plasma membrane; and the microvilli above it. Electron micrograph. Osmium fixation. Buffered osmic acid 1 percent.

show that both the contraction of the muscles and the movement of bacterial cilia is stimulated by adenosinetriphosphate (DeRobertis, Nowinski, and Saez, 1954, pp. 389).

MECHANICAL PROPERTIES OF THE CILIUM

Most of the observations on the structure and and movements of lamellibranch cilia were made on the gills of *Mytilus*. There is no reason to think, however, that the cilia of the oyster gill are fundamentally different from those of the mussel.

The gill cilium is a flexible and elastic rod which can be deformed by mechanical pressure applied with a microdissection needle. The deformity is repaired rapidly when the pressure is removed. Gray (1928) interprets these observations on *Mytilus* cilia as an evidence of transverse elasticity of the cilium.

The movement of the cilium consists of two distinct phases, the forward effective stroke and the much slower recovery stroke which brings the cilium to its initial position. The velocity of the effective stroke is considered to be five times that of the recovery stroke (Kraft, 1890). The effective stroke begins with the curving at the tip and extends down to the base, bending the entire cilium into an arch of 180°; throughout this period the cilium behaves as a rigid rod mounted to the cell by its end. During the recovery stroke the cilium straightens from the base to the tip and moves backward as a limp thread. Both the effective and the recovery strokes take place in the same plane, which remains constant (Gray, 1922a; Carter, 1924).

The movement of a cilium results from contraction of its filaments. It is not clear, however, whether all 11 filaments are equally involved in the effective and recovery strokes. Furthermore, it appears probable, although definite proof is

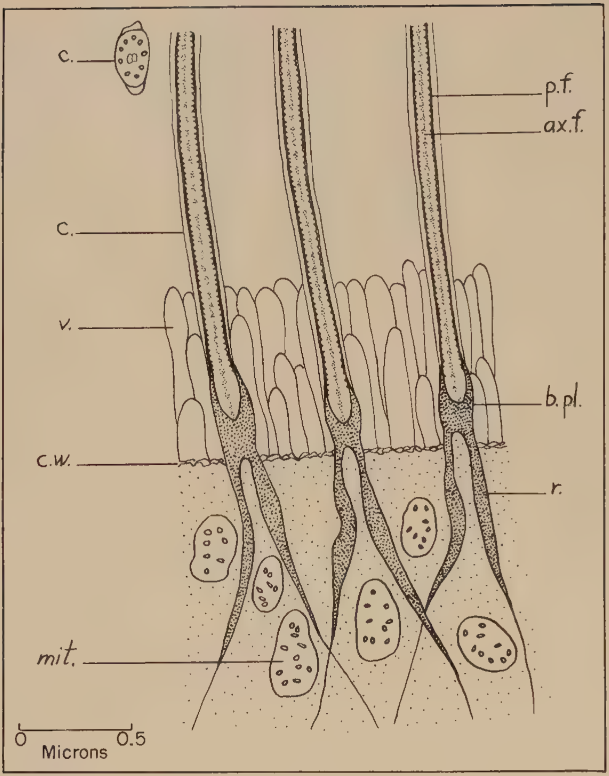

FIGURE 133.—Diagrammatic reconstruction of the distal portion of the ciliated cells of the gill epithelium of *C. virginica.* ax.f.—axial filament; b.pl.—basal plate and basal corpuscle; c.—cilium; cw.—plasma membrane; mit.—mitochondria; r.—rootlet. Cross section of the cilium shown at upper left corner.

wanting, that the pair of axial filaments gives the cilium the necessary rigidity but does not participate in the movement.

METACHRONAL RHYTHM

Automatism is a general characteristic of ciliary motion. This typical property of ciliated epithelium, common to all animals which have ciliated cells, is a fundamental characteristic of the ciliary motion of lamellibranch gills. As Gray (1928, p. 4) stated: "There can be little doubt that all cilia are fundamentally automatic in their movement and that the power possessed by organisms to inhibit the locomotion of their cilia is of extraneous nature."

In any ciliated surface there is some sort of coordinating mechanism that manifests itself in the metachronal rhythm of the beat. The term metachronal rhythm or metachronism denotes the regular sequence of ciliary motion in which any cilium in a given series is slightly out of phase with the cilium behind and in front of it. Since the cilia in one row of the epithelium beat at the same rate but are in different phase, their combined movement gives the optical appearance of a wave passing over a wheat field on a windy day. The beating of the lateral cilia along the isolated filament of an oyster gill is an excellent object in which to observe the metachronal wave. In the drawing of an exposed surface of the gills of a live oyster examined under a compound microscope (fig. 134) the metachronal waves along the two rows of the lateral cilia move in opposite directions. The effective stroke of the lateral cilia in this case is at right angles to the direction of the metachronal wave (i.e., perpendicular to the plane of the drawing). The crest of the wave includes the cilia that are ready to begin their effective stroke; in the troughs are the cilia that are about to start the recovery stroke.

The direction of the metachronic wave is not disturbed by the temporary cessations caused by such extraneous agents as narcotics or cold. Upon recovery the metachronic wave proceeds in the same direction as when the motion was artificially stopped. In the ciliated epithelium of the roof of a frog's mouth the metachronic wave is not disturbed even if a piece of epithelium is cut off and then placed back after rotating it 180° (Brücke, 1917). Transplantation of the gill epithelium of an oyster was tried in the Bureau's shellfish laboratory without success. Copious discharge of mucus, continuous bleeding of the wound area, and the curling up of the filaments interfered with the implantation of the excised pieces. In all my experiments the host animals discarded the implants in a short time.

The fact that small pieces of ciliated surface

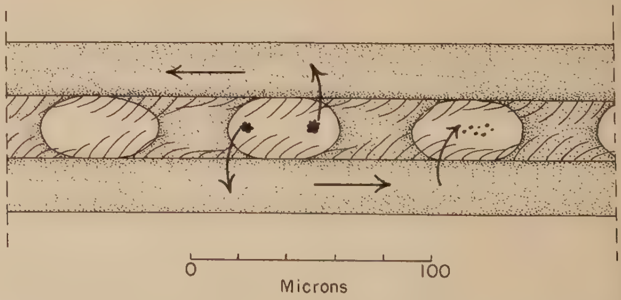

FIGURE 134.—Two tracts of the lateral cilia of *C. virginica* along the two filaments on both sides of the ostia. Small black particles suspended in water are drawn into the ostia while the large ones are discarded by the recovery strokes of the lateral cilia. Drawn from life.

or even single ciliated cells removed from the organism continue to beat for a long time leads to the conclusion that in the majority of cases the ciliary motion is independent of nervous control of the organism. This is, however, not a general rule since the ciliary motion on small fragments of the lips of the snail, *Physa*, removed with the attached nerve, soon ceases unless the nerve is stimulated (Merton, 1923b). Numerous investigations give support to the concept that in many invertebrates and vertebrates the nervous system is an effective agent in the control of coordinated activity of ciliary tracts (Babák, 1913; Carter, 1927; Göthlin, 1920; Lucas, 1935; McDonald, Leisure, and Lenneman, 1927; Seo, 1931).

Bipolar cells and nervelike fibers immediately below the ciliated epithelium of the gills of fresh-water mussels, *Lampsilis* and *Quadrula*, described by Grave and Schmitt (1925), were supposed by these authors to serve as conduction paths for stimuli which they claim regulate and coordinate ciliary movements of the gills of these mollusks. According to their point of view, the ciliated cells of the bivalve gills have a dual control. They may be perfectly autonomous and continue to beat in the complete absence of neural connections; on the other hand, the automatic beat of the cilia may be regulated through supplementary nervous connections in conformity with the state of the organism as a whole. These authors assume that ciliated tissues of fresh-water mussels are both autonomous and under the control of the nervous system.

Intracellular fibrillae of the gills of *Mya*, *Lampsilis*, and *Quadrula* were considered by Grave and Schmitt (1925) to be the conductive paths for coordinating and regulating ciliary movement. A complex system of interconnecting rootlets of the ciliated cells of oyster gills described above (fig. 132) gives additional support to this view. Grave and Schmitt (1925) described also the nervelike apparatus of bipolar cells and fibers. Reinvestigation of the tissues of fresh-water mussels by Bhatia (1926) did not support these findings. No such structures were found in my preparations of the gills of *C. virginica*, or, according to Lucas (1931), in the gills of *Mytilus edulis*. Their existence in the gills of fresh-water mussels seems to be doubtful.

FREQUENCY OF BEAT

The rate of ciliary beat can be observed easily on lateral cilia because of their relatively large size and well-pronounced metachronic wave. Observations must be made on small excised pieces of gill since the position of the lateral cilia on the sides of the filaments makes it impossible to watch their activity on an intact demibranch. In my preparations the filament or a group of them was separated by using fine needles, and kept in a micro-aquarium filled with sea water. The temperature was controlled by circulating cold or warm water in the outside jacket of the microaquarium.

The frequency of beat was determined by using a stroboscope of the type manufactured by R.C.A. and sold under the name "Strobotac". The reddish flickering light given off by this instrument is sufficient to observe cilia under a magnification of about 250 ×. Readings are made directly on the panel of the instrument by rotating the knobs controlling the frequencies. The instrument must be adjusted to the zero point and frequently checked.

Gradual decline in the frequency of beat on the excised filament becomes apparent after several hours; the disturbance of the metachronism in the preparations kept for more than 24 hours is a sign of pathological conditions. Such preparations should be discarded.

The frequency of beat varies greatly in different oysters of the same age, origin, and environment. For instance, among the 12 large adult specimens from New England waters tested in August 1956, the range of variation at room temperature of 22° to 23° C. was from 16 to 27 beats per second. All the specimens were in excellent condition and appeared normal in every respect.

In addition, there are sometimes wide variations in the frequencies of ciliary beat in the adjacent filaments of the excised gills. In studies of the effect of temperature and other environmental factors on the rate of beat, therefore, all the readings must be made over the same portion of the ciliary tract. This is sometimes difficult because of the mobility of the excised pieces and copious secretion of mucus which interferes with the observations.

In the data summarized in table 14 the beat frequencies were recorded in a selected locus of the tract of lateral cilia kept at nearly constant temperature. The filaments were taken from the 14 different oysters listed in the first column of the table. Observations lasted from 10 to 30 minutes. The maximum range of variation recorded during each test was from 16.6 to 20.5 beats per second. The greatest difference between the individual oysters was recorded in two ripe males; one had the median frequency of 15.5 per second (at 23.3° C.) while in the other the cilia beat at the rate of 24.8 per second (at 25.1° C.). In the majority of the oysters the median rate of cilia beat varied between 18 and 22 per second.

TABLE 14.—*Frequency of beat of lateral cilia of 14 adult* C. virginica *recorded at nearly constant temperatures*

[Readings were made at intervals of 1 or 2 minutes]

Oyster	Duration	Temperature range	Beats per second			Recordings
			Max.	Min.	Median	
	Minutes	° *C.*	*No.*	*No.*	*No.*	*No.*
Spawned out, sex undetermined	21	23.8–23.8	20.7	18.3	19.6	20
Two-year-old	10	24.1–24.4	17.3	15.7	16.3	10
Ripe male	15	23.3–23.3	15.3	16.0	15.5	15
Spawned out male	10	23.2–23.6	21.5	20.5	21.2	10
Spawned out male	10	24.0–24.2	21.3	19.3	20.0	10
Ripe male	12	25.1–25.2	25.2	23.5	24.8	10
Spawned out female	30	25.2–25.4	23.7	21.1	22.1	15
Spawned out female	15	24.2–24.3	20.5	16.6	18.5	15
Spawned out female	10	25.1–25.1	20.6	17.3	19.0	10
Ripe female	10	24.5–24.5	18.8.	15.6	17.3	10
Ripe female	20	26.5–26.5	18.0	15.5	17.3	20
Spawned out female	10	23.0–23.1	21.5	19.3	20.7	10
Spawned out female	10	23.0–23.1	19.6.	18.8	19.3	10
Spawned out female	30	22.4–23.2	20.7	19.7	20.6	15

EFFECT OF TEMPERATURE

In evaluating the biological significance of the experimental data of the effect of temperature on beat frequencies, one should remember that the pieces of isolated tissue used were in an abnormal situation. They were deprived of blood supply, separated from close association with other structural elements of the gill, and subjected to increased concentrations of metabolites. It is conceivable that under normal conditions the lateral cilia of an intact gill may react somewhat differently.

Stroboscope observations fully confirm the fact that temperature controls the ciliary beat. This effect was observed in a series of determinations made during the summer using small pieces of filaments taken from 39 adult New England oysters kept in water at various temperatures. At the start of each series of readings 10 minutes were allowed for adjustment to the desired temperature which was kept constant within plus or minus 1° C. Ten stroboscope readings were made at 1-minute intervals and repeated at higher or lower temperature. No more than three different temperature levels were used on one preparation. Careful precautions were taken to prevent the movement of the excised filaments in the microaquarium so that all the readings would be made on exactly the same locus of the ciliary tract. This was necessary because of the considerable differences in the rate of beating which occasionally occur along the adjacent filaments.

The results, summarized in table 15, show the maximum median frequency of 27.7 beats per second at temperatures of 25° to 27° C. The ciliary activity became irregular at about 35° C., and the movement ceased at 37° to 38° C. Whether these limits are applicable to oysters from warm southern waters is not known, since all the observations were made only on the New England oysters. Between 35° and 37° C. the motion was so irregular that its frequency could not be recorded with certainty. Irregular beating at the rate of about two beats per second was observed in some specimens during short exposure to the temperature of 45.6° C. Judging by the median values of the beat frequencies, the optimum temperature is between 23° and 27° C. (see fourth column, table 15). The ciliary activity declines rapidly below 21° C. and ceases completely at 5° to 7° C.

Individual variations in the frequency of beat among oysters of a single population suggest differences in their physiological states and different requirements for food and water for respiration. As a rule, spawned-out females remained inactive for some time in late August and early September. During this period the gonads containing unspawned sex cells were reabsorbed and tissues became watery because of the reduction in solids content. The adductor muscles remained contracted, and the shells were closed for unusually long periods, lasting from 3 to 4 days, or opened

TABLE 15.—*Frequencies of beat of lateral cilia of the gills of adult* C. virginica *at different temperatures*

[Stroboscope readings made on excised filaments kept in sea water]

Temperature range	Frequency of beats per second			Preparations used	Oysters used
	Minimum	Maximum	Median		
° *C.*	*Number*	*Number*	*Number*	*Number*	*Number*
36–37	Irregular	Irregular	Irregular	2	1
33–35	----------	24.2	----------	1	----------
31–33	17.3	27.7	22.5	5	2
29–31	23.8	23.8	23.8	1	1
27–29	19.3	27.7	23.3	3	2
25–27	20.8	33.3	27.7	7	3
23–25	21.3	33.3	25.6	7	3
21–23	16.6	27.7	20.8	13	5
19–21	16.7	20.3	17.2	3	2
17–19	13.3	16.7	13.7	6	2
15–17	10.0	11.6	10.5	6	2
13–15	3.6	11.9	10.2	7	3
11–13	2.2	8.9	5.6	8	3
9–11	1.6	2.6	2.1	8	2
7–9	1.5	1.9	1.8	5	2
5–7	----------	----------	----------	5	5

only for a short time. Even when the valves opened, the gills produced a weak and unsteady current interrupted by frequent cessations of ciliary motion.

The effect of temperature on ciliary activity can be seen more clearly in the experiments in which only a single gill filament was used. The results are shown in figure 135 in which the median frequencies of the beat are plotted against the temperature. As in previous observations 10 readings were made at each temperature step and the entire experiment was completed in about 2½ hours. The frequency of beat rapidly increased between 10° and 25° C. The slowing down of ciliary motion below 10° C. was gradual until all movements ceased at about 6° C. The curve shown in figure 135 has four distinct slopes that indicate the differences in the response of the lateral cilia to temperature changes: a) a very slow increase between 6° and 11° C.; b) a more rapid acceleration between 11° and 15° C.; c) a gradual increase between 15° and 25° to 26° C.; and d) a decline as the temperature rises toward the 30° C. mark.

COMPOSITION OF SEA WATER AND CILIARY MOTION

Ciliary motion may be affected by changes in the chemical composition of sea water and by various drugs. Ionic balance of the outside medium is one of the principal conditions for continuous ciliary activity of the gill. The most important ions are sodium, potassium, calcium, and magnesium; the increase in concentration of one without a corresponding compensation in the concentration of another or the withdrawal of one of the ions may completely disrupt the ciliary motion.

EFFECTS OF CHEMICALS ON CILIARY MOTION

METALLIC IONS

The most favored object for study of the effect of ions on ciliary motion of bivalve gills has been the frontal cilia of the excised pieces of *Mytilus* gills (Lillie, 1906; Gray, 1922b). Only occasionally were the lateral cilia used in these observations.

The monovalent metallic ions are important in the stability of the ciliated cells and maintenance of ciliary motion. By using a series of samples of artificially varied sea water it can be shown experimentally that the replacement of sodium by other monovalent cations rapidly affects the rate of ciliary beat. The effect is the greatest with lithium and smallest with potassium. In the order of their effectiveness the ions can be placed as follows: Li< Na< NH_3< K. There is, however, a marked difference between the effects produced by sodium and potassium. The frontal cilia beat more rapidly in solutions containing greater concentrations of potassium and are less affected by changes in the concentration of sodium. The laterofrontal cilia of *Mytilus* are affected by potassium in a manner not observed in other cilia. The first reaction to the increased concentration of this ion is an increase in the rate of beating. With further addition of potassium the recovery stroke becomes incomplete and the cilia vibrate very rapidly with greatly reduced amplitude and impaired efficiency.

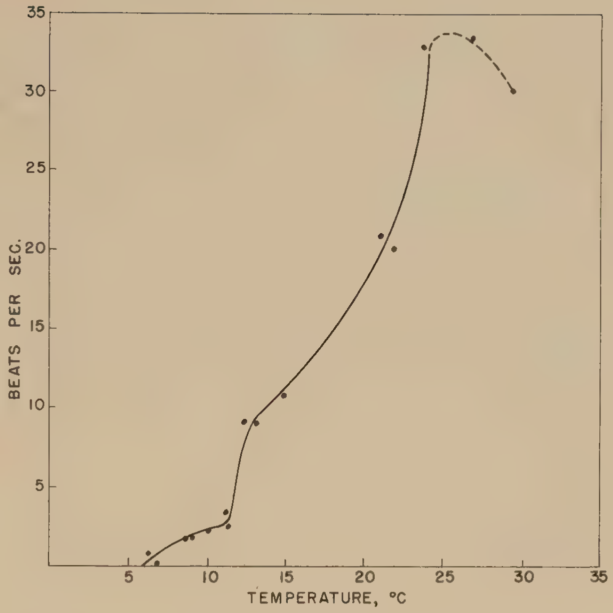

FIGURE 135.—Effect of temperature on the median frequencies of beat in number per second of the lateral cilia of *C. virginica*. Readings were made with Strobotac on a single filament of the gill kept in sea water in a microaquarium. Temperature was changed by circulating warm or cold water in the jacket of the microaquarium. Duration of the experiment 2½ hours.

Magnesium inhibits the beat of the lateral cilia of the excised pieces when the concentration of this metal in the surrounding water exceeds its concentration in the blood. Potassium antagonizes the action of magnesium while sodium produces no such effect.

The difference between the effects of magnesium and potassium is also apparent in the way these ions act on the stability of the intercellular matrix. Under normal conditions magnesium is essential for the maintenance of stability. If the gill preparation is placed in a medium containing sodium and magnesium, the cells remain stable; these deteriorate rapidly in a mixture of magnesium and potassium. It is probable that the potassium ion drives away magnesium from certain areas inside the cell and sodium ions do not (Gray, 1922b). In the absence of calcium the rate of ciliary beat is gradually decreased and eventually ceases (Gray, 1924), but the increase of calcium in the surrounding water produces no marked effect on ciliary motion.

As long as the normal equilibrium of the cations sodium, potassium, calcium, and magnesium is maintained in the surrounding medium, the ciliated cells (of *Mytilus*) are insensitive to changes in the concentration of anions (Cl^-, NO_3^-, Br^{--}, I^{--}, acetate, and SO_4^{--}).

It may be assumed that the results of observations on *Mytilus* gills are applicable to the oyster and that changes in the ionic equilibria in sea water may have a similar effect on the efficiency of the ciliated mechanism of oysters.

HYDROGEN IONS

The effect of variations in the concentration of hydrogen ions on the rate of ciliary motion in bivalve gills is greater than that caused by changes in the concentrations of any other ions. This has been demonstrated on the gills of *Anodonta, Mytilus, Mya,* and *Ostrea* (Chase and Glaser, 1930; Gray, 1928; Haywood, 1925; Nomura, 1934; Yonge, 1925). The greatest effect is produced by those acids which, like carbonic acid, penetrate the cell surface most rapidly. Nomura (1934) found the following order of efficiency of acids in arresting the ciliary motion of *Pecten:* $H_2CO_3 > CH_3COOH > H_3PO_4 > HCl$. Ciliary activity ceases in 1 minute at pH 3.8 when HCl has been added, but with CH_3COOH or H_2CO_3 the stoppage would occur in the same time at the much higher pH of 5.5. A decrease in the pH values of sea water from 8.1 to 6.1 reduces the ciliary motion of the gills of *C. virginica* to about 37 percent of their normal rate. In these observations by Galtsoff and Whipple (1931) the pH of sea water was changed by bubbling carbon dioxide, and measurements were made of the rate of flow of water produced by the lateral cilia. Ciliary motion stops completely over the entire gill surface of the oyster when the pH of water is reduced to 5.3 to 5.6. Minimum pH in which the cilia can function depends on the concentration with which they are normally at equilibrium. This was demonstrated clearly by Yonge (1925) on the cilia of *Mya*. Thus the average pH inside the style sac of this clam is 4.45 and the cilia of the sac stop functioning below pH 3.5 to 4.0, while the gill cilia normally surrounded by sea water of about pH 7.2 come to a standstill at pH 5.2 to 5.8.

VARIOUS DRUGS

The effects of various drugs on ciliary motion of the gill epithelium of *Anodonta, Pecten, Mytilus,* and *Ostrea* have been observed by various investigators.

The reaction to any effective drug usually takes place in four consecutive stages: (1) retardation of the frequency of beat, (2) disappearance of metachronism along the ciliary tract and its perseverance within the individual cells (unicellular metachronism), (3) synchronous beating of the cilia of a single cell (disappearance of unicellular metachronism), and (4) cessation of beat.

The degree of depression depends on the concentration of the drug used and the duration of its action. Cessation of beat in the gills of *Anodonta* was observed in the following compounds (Bethell, 1956): 0.5 percent chloral hydrate (in 4 to 5 minutes); 1 percent novocaine (9 minutes); 1.5 percent pilocarpine hydrochloride (in 10 minutes). In 1 to 1.5 percent veratrine sulfate the metachronal wave slows until movement ceases. Caffeine (2 percent solution) accelerates the ciliary motion for 3 minutes and in 6 minutes completely depresses it. The effect of adrenaline on the gills of *C. gigas* was studied by Nomura (1937). The rate of ciliary motion was observed on excised oblong pieces of the gill that were placed in a graduated, narrow glass tubing. They crawled along the glass surface of the tubing, and their advance during 1 minute was recorded. The crawling velocity in various concentrations of adrenaline also was recorded, and the degree of depression of ciliary motion was expressed in percentage of the velocity attained in natural sea water. The results show that the ciliary movement is depressed in proportion to the concentrations, which varied from 10^{-10} to 10^{-5}.

Observations made in the Bureau's shellfish laboratory at Woods Hole using adult *virginica* showed that 5 ml. of 1 percent solution of chloral hydrate applied to the mantle cavity of an oyster kept in a 4 l. tank of slowly changing water depressed the beating of the lateral cilia by 50 to 87 percent. Twenty-five minutes after the removal of the drug the effect disappeared and normal (i.e., preceding) rate of ciliary motion was reestablished. Application of 1 ml. of 0.1 percent chloral hydrate to the mantle and gills had no visible effect, but 4 ml. of the same concentration injected in the vicinity of the gills increased the ciliary motion by 15 percent. The effect lasted only a few minutes.

In the above experiments the duration of the drug action was brief since the oysters were kept in running sea water. Different results were obtained when the test oysters were left in stagnant water. No appreciable effect was noticed in 0.015 percent solution of chloral hydrate, a slight decrease (about 12 percent) was recorded in 0.019 percent, and the ciliary action stopped in 0.03 percent solution.

Slight depression of ciliary motion (from 11 to 13 percent) was obtained by a single 1 ml. dose of nembutal solution (concentration 0.02 g. per l.) injected directly into the mantle cavity. No decrease in ciliary motion appeared in the control tests in which 1 ml. of sea water was injected. Ciliary activity in all these tests was measured by determining the velocity of the cloacal current.

Introduction of 3 ml. of digitalin (1:500) into the pallial cavity results in an immediate, 90 percent depression of ciliary activity. Figure 136 represents part of the record obtained by using the electric drop counting method described in chapter IX, p. 190. The effect is dissipated in about 2 minutes.

A solution of pilocarpine of 1:10,000 in sea water applied directly to the excised pieces of *C. virginica* gills has no effect on lateral cilia. In the test made in the Woods Hole laboratory the frequency of beat in natural sea water varied in this experiment from 10.5 to 11.4 per second, and from 10.3 to 10.9 per second after addition of the drug. The concentration of 0.5 percent slowed down the frequency by approximately 40 percent (6.5 to 6.8 per second). All observations were made at 23.5° C. Atropine sulfate solution of 1:1,000 had only a slight effect on the frequency of beat of the lateral cilia, reducing it by about 17 percent at 22.3° C.

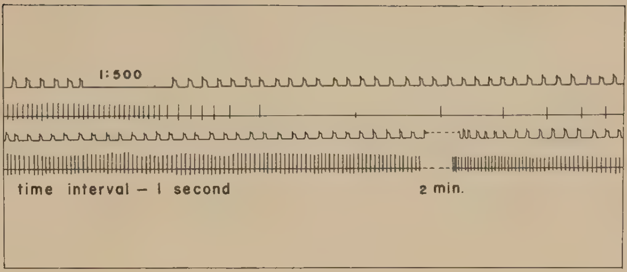

FIGURE 136.—Kymograph record of the effect of digitalin (1:500) on the rate of ciliary activity of the gill of *C. virginica.* Electric drop counting method. First and third line indicate time intervals of 1 second; dotted line marks the 2 minute interruption in recording. Second and fourth lines show the contacts made by each drop of water discharged through the cloaca. Two ml. of digitalin solution were injected into the pallial cavity in 5 seconds, which are indicated at the top by the straight line which interrupts the beat recording. A signal key was depressed for 6 seconds (upper line) when the digitalin was being added.

The effects of acetylcholine and eserine are of particular interest because of their importance to the functioning of the neuromuscular mechanism. Eserine inhibits the action of choline esterase, the enzyme which hydrolizes acetylcholine and prevents its accumulation. The latter would cause an excessive neuromuscular activity. Nomura and Kagawa (1950) found that at concentrations higher than 10^{-6} both acetylcholine chloride and eserine inhibit ciliary movement of the gills of *C. gigas.* These investigators deduced from their observations and from the experiments of Nomura (1937) that acetylcholine and adrenaline, while inhibiting ciliary motion in the oyster, have the opposite effect on the heart of this mollusk.

INHIBITION OF CILIARY MOVEMENT BY ANTISERUM

Antiserum produced in rabbits by the injection of minced gill tissue of *Anodonta* inhibits ciliary motion of the gills of this species. This observation of Galli-Valerio (1916) was confirmed by Makino (1934) for *C. gigas, Meretrix,* and other bivalves.

The problem was further studied by Tomita (1954, 1955), who improved the technique of preparation of the antisera by eliminating the preservatives (merthiolate and phenol) which are known to depress the ciliary motion in the concentrations commonly used for this purpose.

The antigens were prepared by Tomita in the following manner. The gills of *C. gigas*, *Anadara inflata*, and *Pecten yessoensis* were minced in 0.85 percent saline and homogenized in a blendor. The protein content of the homogenate was estimated from the determination of nitrogen made by microKjeldahl method, and the preparation was diluted with saline to give the final protein content of 1 mg. per ml. Merthiolate in the concentration of 1:10,000 was added as a preservative. On alternate days a quantity of antigens containing 2.5, 5.0, and 7.5 mg. of protein per kg. of body weight were injected into healthy rabbits. After 2 weeks the animals were bled and the antisera were placed aseptically in sterile ampules without any preservatives and stored in a refrigerator.

Small pieces of gill tissues, 3 to 4 mm. long and 3 mm. wide were cut from the free margin of the middle demibranch and placed in sea water in a glass tubing about 12 mm. in diameter. The relative speed of crawling estimated by Nomura's method (1937) was taken as a measure of ciliary activity in normal sea water (100 percent efficiency) and in various dilutions of the antiserum. Complete stoppage of crawling was recorded in the dilution 1:40 after 32 minutes. Considerable depression of ciliary motion was noticed in the dilution 1:320 after 77 minutes of exposure. It is regrettable that no observations were made on the ciliary motion of an intact gill or that the frequency of ciliary beat in the excised pieces was not measured by a stroboscope or by any other technique more reliable than the "crawling" method.

The antisera of the two other species of bivalves (*Anadara* and *Pecten*) have an inhibitory effect on the gills of *Ostrea*. The inhibition was, however, less pronounced than that caused by the anti-*Ostrea* serum. The anti-muscle serum tested on the gills of all three species was less effective than the anti-gill serum. The author deduced from these observations that both "tissue-specificity" and "species-specificity" are involved in the inhibitory effect of the antisera.

EFFECT OF PRESSURE ON CILIARY MOTION

Observations of the effects of increased hydrostatic pressure on ciliary motion were made by Pease and Kitching (1939) using the gills of *Mytilus edulis*. Part of an excised gill plate was placed inside the glass chamber of a pressure bomb designed by Marsland, and the surrounding sea water was saturated with veratrine, which according to Gray (1928) considerably prolongs the activity of the cilia.

Under normal pressure the rate of beating, measured stroboscopically, was about 9 to 10 times per second, considerably slower than the normal rate of 15 to 17 per second that one expects at the temperatures of 21° to 24° C. at which the tests were conducted. Apparently the use of veratrine was unnecessary because the duration of the experiments did not exceed 90 minutes and some of them were completed within 8 to 16 minutes. The tests show that a sudden increase in the hydrostatic pressure by 1,000 pounds per square inch or more immediately increases the frequency of beat of the lateral cilia. Decompression results in a reduction in frequency below the normal level and slow recovery. Pressure in excess of 5,000 pounds per square inch decreases the frequency and causes permanent injury. The authors claim that the change in temperature due to compression or decompression is too small to account for the observed effects, because, on theoretical grounds, it may be expected that the temperature increases by 0.6° C. when the water is compressed adiabatically to 5,000 pounds. The actual temperature in chamber of the pressure bomb was not observed.

It would be of interest to repeat these experiments using pieces of gill epithelium kept in normal sea water not poisoned by veratrine.

CILIARY CURRENTS OF THE GILLS

The ciliary currents at the surface of the gills of an intact organ can be observed by dropping small particles (carmine, carborundum, colloidal carbon, and willemite) on the surface of the demibranchs and following under the binocular microscope their movement and direction. The most important contributions to the studies of this subject were made by Wallengren (1905a), Orton (1912), Kellogg (1900, 1915), Yonge (1926), and Atkins (1937, 1938).

There are five major tracts on the surface of the gill of *C. virginica* (fig. 137). The frontal cilia beat parallel to the surface of the demibranch from the base toward its free margin. This current, maintained along all ordinary filaments (or.f.), carries the particles settled on the surface of the gill to the terminal groove (tr.g.). This is lined with ciliated cells that beat parallel to the edge of the gill and push the particles entangled in mucus toward the mouth. Between the plicae the current caused

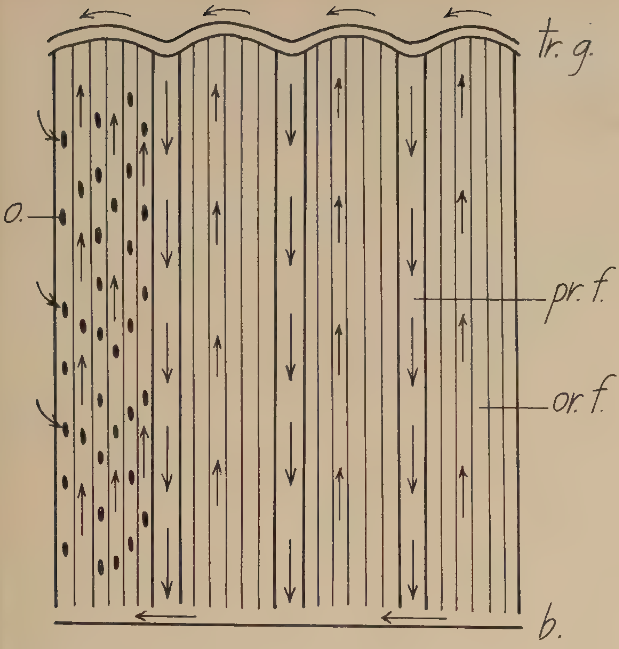

FIGURE 137.—Diagram of the system of ciliary currents on the surface of the demibranch of *C. virginica*. The four plicae are shown slightly pulled apart to indicate the principal (wide) filaments at the bottom of the grooves. Open ostia, o., are shown only on the left plicae; the mouth is toward the left; b.—base of the gills; or.f.—ordinary filaments; o.—ostia; pr.f.—principal filament; tr.g.—terminal groove.

by the frontal cilia of the principal filaments (pr.f.) runs in the opposite direction, i.e., from the free edge of the gill toward the base. Particles carried by this current enter the track along the base of the gills (b.), which runs parallel to the direction of the current in the terminal groove and carries food particles toward the mouth. The lateral cilia (not shown in the diagram) beat at right angles to the surface of the gill and create a current that forces water inside the water tubes and into the epibranchial chamber.

Small single particles fall into the grooves and eventually are carried by the principal filaments toward the mouth while the larger particles or a mass of small ones entangled in mucus are pushed by the frontal cilia toward the free edge of the demibranch and may be dropped from the gill before entering the terminal groove. Frequently a group of particles is passed from the edge of one demibranch to the surface of the underlying one before it is discarded. The complex system of ciliary currents in the gill constitutes an efficient selective mechanism for the sorting of food. Final selection is made along the surface of the labial palps, which reject a large portion of the material brought in by the gills (see p. 115)

The ciliary tracts of the gills of *O. edulis* described by Atkins (1937), in general resemble those observed on the gill of *C. virginica* (fig. 138). In the three species of oysters *C. virginica*, *O. edulis*, and *C. angulata*, the ciliation is essentially the same.

MECHANICAL WORK OF THE LATERAL CILIA

The lateral cilia function principally as movers of water. They force water through the ostia into the water tubes of the gills and maintain inside the gill a current that passes through the branchial chambers to the outside. The hydrostatic pres-

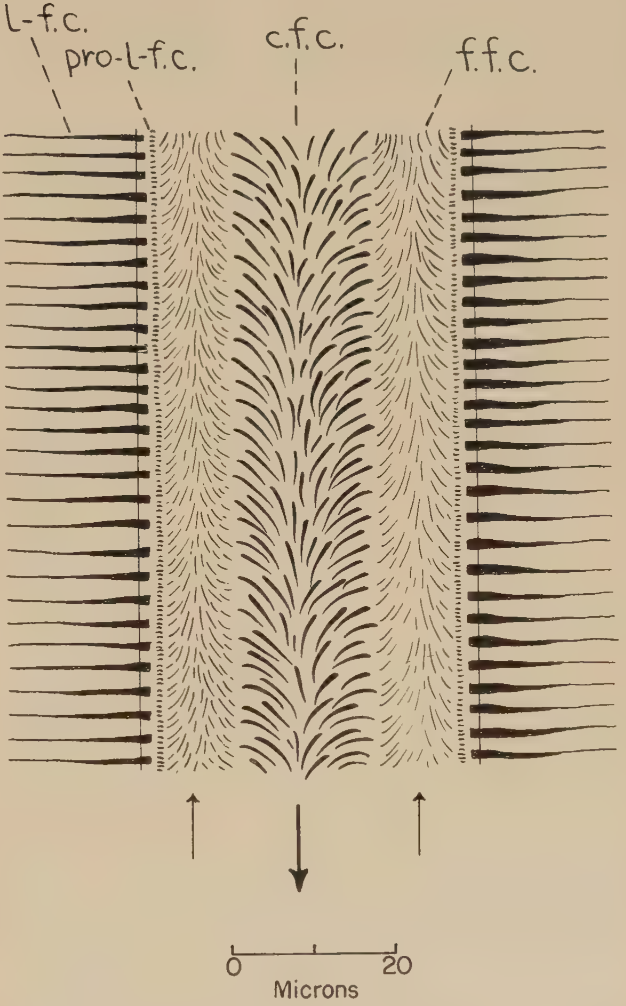

FIGURE 138.—Frontal view of a living filament of *O. edulis*. c.f.c.—coarse frontal cilia; f.f.c.—fine frontal cilia; lf.c.—laterofrontal cilia; pro.lf.c.—subsidiary laterofrontal cilia. From Atkins, 1937, figure 1.

sure inside the gill chamber is maintained solely by these lateral cilia, which form a pumping mechanism with their synchronized beating over the entire gill surface.

Local disturbance in the coordination of ciliary motion caused by the change in the ratio between the effective and recovery strokes or by the changes in the phase of beat results in a drop of pressure and decrease in the current velocity. In the absence of valves or any other regulatory devices, the synchronous beat of the lateral cilia over the entire surface of the gills is an essential condition for the effective functioning of the gill.

One can see under the microscope that slight mechanical disturbances, such as tapping of the dish in which the gill fragments are kept, disorganize the metachronal wave of the lateral cilia and affect the frequency of their beat. The gill may be compared to a folded tubular sieve, with the meshes of the sieve corresponding to the ostia surrounded by the lateral cilia. The contraction of the gill muscles brings the filaments together, constricts the ostia, and reduces the spaces between the filaments. In this way the passage of water through the gill may be restricted.

CARMINE CONE METHOD

The efficiency of the lateral cilia can be measured with a simple device known as the carmine cone method (Galtsoff, 1926). The method is based on measurements of the velocity of the gill's current in a horizontal glass tubing introduced into the cloaca. The valves of the oyster are gently forced apart until they are wide enough to allow the insertion of soft rubber tubing into the cloaca. A wooden wedge is placed between the valves to keep them from closing. The insertion of rubber tubing of a suitable diameter is made by gently rotating it counterclockwise until the rubber is slightly pressed against the outside wall of the cloaca. The tubing is then secured in its position by packing the space around it with cotton. A cotton plug is inserted into the opening of the promyal chamber and is covered with plastic clay. The entire operation can be performed within 2 or 3 minutes and is greatly facilitated by narcotizing the oyster in an 8 to 10 percent solution of magnesium sulfate in sea water.

The oyster with rubber tubing in the cloaca is then placed in a shallow white enamel tray filled with sea water and gently tilted back and forth to remove any air bubbles that may have remained under the valves. A small balloon pipette is introduced into the rubber tubing to suck out the air bubbles that may be trapped in the epibranchial chamber. The presence of the cloacal current is checked by placing a drop of fine carmine suspension against the end of the tubing. The suspension may be added to the gills as well, and in a few seconds a fine carmine cone appears in the cloacal current.

The end of the rubber tubing is now connected to one arm of an inverted T tube which has a slightly curved glass funnel sealed inside the other arm. This arm is joined to a horizontal glass tubing of known diameter, not less than 15 cm. long and graduated in 0.5 cm. (fig. 139). A thistle funnel filled with fine carmine suspension is attached so the vertical arm of the inverted T tube, and the tube and the funnel are held by two clamps mounted on a heavy stand (not shown in the diagram). The carmine suspension must be released by a pinchcock without disturbing the rubber tubing inserted in the cloaca, and the amount released must be very small in order to avoid back pressure of water in the gills. Because of the frictional resistance of water moving inside a circular tube, the highest current velocity is at the center of the cross sectional area of the horizontal tube. A minute quantity of carmine suspension or of a solution of nontoxic dye in sea water released from the funnel forms a sharply defined cone inside the tube, the tip of which moves from zero to 10 or 15 cm. mark; the time of its

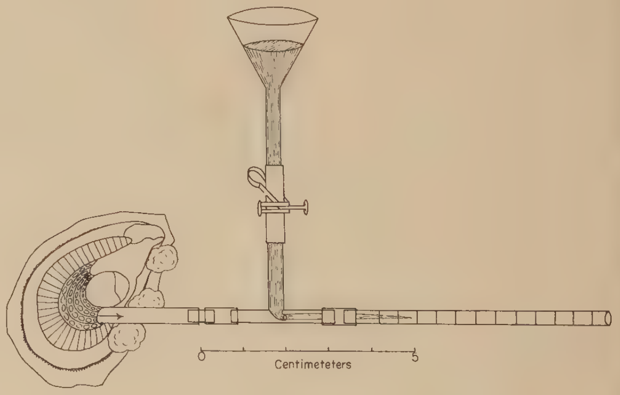

Figure 139.—Diagram of the carmine cone method for the study of the efficiency of the lateral cilia of the oyster gill. In order to indicate the position of rubber tubing inside the cloaca, the right valve is not shown; the tank in which the oyster is kept is omitted from the diagram. The funnel with carmine suspension is perpendicular to the plane of the drawing.

passage is recorded by using a stop watch graduated to one-tenth of a second.

Glass tubing of sufficiently wide diameter should be used to avoid turbulent flow. For large *C. virginica* tubing of 5 to 6 mm. in diameter was satisfactory.

The efficiency of the lateral cilia can be expressed either in terms of the velocity of the cloacal current or by computing the mechanical work they perform. The fact that a distinct cone forms at the center of the tube through which the current is running indicates that we are dealing with a viscous flow for which the velocity can be expressed by the Poisseul's formula:

$$S=\frac{D^2\Delta p}{16\mu l}$$

In this formula S is the velocity at the axis of the tube in cm./sec.; D is the diameter; and l the length of the tube in cm.; $\Delta\ p$ is pressure drop between the two marks along the tube in dynes/cm.^{2}; and μ is viscosity of sea water in poises (C.G.S. unit).

The mean velocity (Sm) of the current of the entire cross sectional area of the tube is one-half the velocity at the axis. The rate of discharge, V, in cc. per second is computed by using the following formula:

$$V=\frac{\pi D^2 0.5S}{4}$$

The rate of mechanical work W (in ergs per second) can be determined from the formula: $W=2\pi l\mu S^2$. For a detailed discussion of the mechanical activity of oyster gills, the reader is referred to the original publication of Galtsoff (1928b).

The cone method is simple and requires no elaborate equipment. It can be used in any field or temporary laboratory and is particularly useful for rapid toxicity tests in tracing the physiologically active components of various pollutants. The method has, however, several limitations that should be kept in mind. First, the volume of water passing through the cloaca does not represent the total amount transported by the gills because a certain portion of the water is discharged through the promyal chamber. Second, the tests should be completed in 1 day because the prolonged presence of tubing inside the cloaca and of the wedge between the valves may produce pathological conditions. Because of the sensitivity of the cilia to mechanical disturbance great care should be exercised to avoid jarring, shaking, and vibrating when preparing a test. The oysters usually recover within 12 hours after being placed in running sea water and show no ill effects of the narcosis and handling.

EFFECT OF TEMPERATURE

The cone method proved satisfactory in a study of the effect of temperature on the efficiency of the lateral cilia. The results of many tests performed in the Woods Hole laboratory show considerable variability in the velocity of the cloacal current of oysters of the same size and origin. At a given temperature and under identical conditions the lateral cilia of some oysters work faster than those of others. Consequently, no definite rate of work maintained by the gill epithelium at a specified temperature might be considered as typical or normal for an oyster of a stated size and type.

An example of the effect of temperature on current velocity produced by the lateral cilia of oysters of identical size transporting water at different rates is shown in figure 140. In both experiments the water was agitated by an electric stirrer and its temperature was changed by using heating or cooling units placed at the end of the tank farthest from the oyster. Not less than 15 minutes for adjustment was allowed at each temperature step. Readings were made starting at 20° C. and decreasing to the extreme low temperature at which no current was produced. Then the water

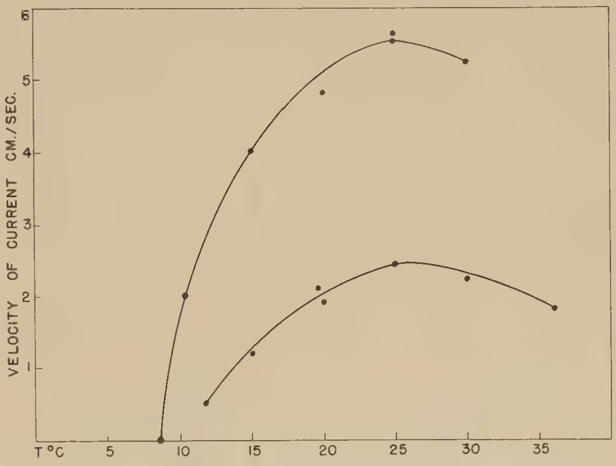

FIGURE 140.—Effect of temperature on the velocity of the cloacal current produced by slow (lower curve) and fast (upper curve) adult oyster, *C. virginica*, of about 4 inches in height. Each dot represents the mean velocity of the current of 10 consecutive readings made at intervals of 2 to 3 minutes. Carmine cone method.

was warmed to the extreme high and cooled again to 20° C. for the last observation. Each circle represents a mean of 10 consecutive readings made at intervals of 2 to 3 minutes. The lower curve represents the activity of an oyster in which slow ciliary motion started only at 11.3° C. The upper curve is typical for an oyster which maintains a rapid transport of water. In both curves the maximum activity occurred at 20° to 25° C. Rapid acceleration in the rate of current took place between 10° (or 11.3°) and 15° C. Essentially the relationship between the temperature and current velocity is similar to the effect of temperature on the frequency of beat of lateral cilia shown in figure 135, although the slope of the latter curve is steeper than in the two curves shown in figure 140. Within the range of the temperature used in these tests, the action of the cilia was completely reversible.

The increased rate of activity induced by temperature may be expressed by temperature coefficients determined at 10° intervals. These values, calculated from a large number of observations with the cone method and given in table 16, show considerable difference in Q_{10} based on the determinations of current velocity and on the rate of work.

Table 16.—*Temperature of coefficients* (Q_{10}), *of the rate of ciliary activity of lateral cells of C. virginica*

Temperature range	Temperature coefficient based on velocity of current	Temperature coefficient based on rate of work performed by the cilia
°*C.*		
5–10	6.0	4.8
10–20	2.5	4.4
15–25	1.8	2.4
20–30	1.5	1.8
25–35	1.3	1.2

The current velocity is not a true measure of the work performed by cilia because the viscosity of the water changes with temperature. In the formula $W=2\pi l\mu S^2$ the work required to maintain a current at a constant speed is proportional to viscosity, μ. Since at a lowerte mperature the viscosity of sea water is greater than at higher temperatures, more energy is required to propel cold water. As the work needed to produce current of a given velocity is proportional to the square of the velocity at the axis of the current, it is apparent that the decrease in the frictional resistance due to lesser viscosity of water is not sufficient to compensate for the additional energy required for maintaining faster current. Temperature coefficients computed on the basis of the rate of work performed are, therefore, more significant than the Q_{10} based on the velocity of current.

HYDROSTATIC PRESSURE INSIDE THE GILLS

The velocity of the cloacal current is proportional to the difference in hydrostatic pressure inside the gill chambers and at the opening of the cloaca. The pressure can be measured by introducing an L-shaped glass tubing into the free end of the rubber tubing inserted into the cloaca and recording the difference between the level of sea water in the tube and the level in the container in which the oyster is kept. Correction should be made for the position of the meniscus in the tube due to surface tension. Using this simple device I found that in an actively feeding adult *C. virginica* the pressure inside the epibranchial chamber may be as high as 7 to 8 mm. of sea-water column. If the temperature and salinity of water are known, the pressure may be calculated in grams per unit area.

SPONTANEOUS INHIBITION OF CILIARY MOTION

When the bivalve mollusks close their shells and cut off their access to outside water, they enter a state of suspended animation during which their normal functions are greatly slowed down or completely cease. This state of diminished activity observed in *Anodonta* and *Sphaerium* (*Cyclas*) was regarded by Gartkiewicz (1926) as sleep. Through the transparent shell of *Sphaerium* he was able to see that the ciliary motion of the gills and the beating of the heart were at a complete standstill when the shells were closed. This observation corrected the erroneous opinion of earlier investigators (Wallengren, 1905a, 1905b) that ciliary activity persists when the valves are closed.

The cessation of ciliary motion after the closing of shells was attributed to the accumulation of carbon dioxide and the decrease of pH. A pH of less than 6.0 probably does not occur in the body fluids of bivalves after they close their valves because of the buffering action of carbonates of the shell substance.

In the gills of *C. virginica* ciliary motion ceases shortly after the closing of the valves and is renewed after they open. It is probable that in these cases the depression of ciliary activity is due primarily to the accumulation of metabolites There exists, however, another type of inhibition of ciliary motion that is not associated with changes in the outside environment. It can be observed on gills exposed by the removal of a portion of the valve. The oyster is placed in a suitable container supplied with slowly running sea water, and the gills are strongly illuminated and examined under a dissecting microscope.

The time required for a small inert particle (carmine, or powered oyster shell) to be moved along the terminal groove between the two selected points in the microscope's field of view is recorded with a stopwatch. Copious discharge of mucus that impedes the transport of particles along the groove was avoided by adding only minute quantities of material in suspension. Readings were repeated every minute, and the degree of expansion of the gill lamellae and ostia were recorded. The observations lasted from 10 to 30 minutes. Ciliary motion over the terminal groove of the gill frequently slowed down as the adductor contracted, but previous rhythm was resumed within a few seconds after relaxation of the muscle. The most spectacular were the instances of complete cessations of ciliary motion over the surface of the entire gill following strong contraction of the adductor muscle and complete closure of the valves. Since a portion of the shell was removed the surface of the gill remained in contact with fresh sea water and the cessation of ciliary activity could not be attributed to the accumulation of carbon dioxide or other metabolites.

The association of the inhibition of ciliary motion with the contracted state of the adductor muscle is shown in table 17, which contains excerpts of the records of observations made on two male and two female adult oysters. Temporary depression and sometime stoppage of ciliary motion were often observed after occasional contractions of the gill muscles. In these cases the inhibitory impulses seem to be less pronounced than in the case of the contraction of the adductor muscles. Electric shock applied from the DuBois inductorium direct to the gill epithelium or to the edge of the mantle had no effect on ciliary beat of the frontal and terminal cilia. Only in the case of a shock sufficiently strong to cause contraction of the adductor muscle was there a cessation of ciliary activity.

TABLE 17.—*Association of the velocity of ciliary current along the terminal groove of the external right demibranch and the state of contraction of the adductor muscle*

[Excerpts of the protocols of the four experiments with adult and sexually mature C. virginica made in August at Woods Hole, Mass.]

Sex	Time [1]	Temperature	Adductor	Time needed to move a particle over a distance of 1 cm.	
				Min.	Max.
		°*C.*		*Seconds*	*Seconds*
Female	7:42– 7:44 a.m.	21.6	Relaxed	13.6	13.8
	7:45– 7:47 a.m.	21.6	Contracted	22.0	22.0
	7:48 8:10 a.m.	21.6	Relaxed	12.0	12.5
Male	10:20 10:22 a.m.	21.0	Relaxed	22.0	23.0
	10:24–10:27 a.m.	22.0	Partially contracted.	26.6	29.2
	10:30–10:35 a.m.	22.0	Relaxed	22.3	22.6
Female	4:58– 5:08 p.m.	21.5	Relaxed	20.6	22.2
	5:25– 5:33 p.m.	22.0	Contracted [2]	no movement	
Male	3:21– 3:28 p.m.	22.8	Relaxed	14.4	16.6
	3:31– 3:41 p.m.	22.8	Contracted	no movement	

[1] Readings made every minute within time shown in this column.

[2] Complete contraction. Ciliary motion stopped along the entire terminal groove and on the surface of the gill.

Extirpation of the visceral ganglion or its burning with an electric needle had no effect on ciliary motion of the gill, indicating that inhibition does not originate in the ganglion. The frequent coincidence of the cessation of ciliary motion with the contraction of the adductor muscle and the subsequent resumption of ciliary activity after its relaxation suggests the possibility of a neuroid transmission of the inhibitory impulse which may originate during muscular activity and spread over the ciliated surface of the gill.

Since the problem of the impulses causing inhibition of ciliary motion has not been studied sufficiently, it is impossible at this time to present a reasonable explanation of this puzzling phenomenon.

The transport of water by the gills during feeding and respiration is discussed in chapter IX since this function is controlled jointly by the mantle and adductor muscle.

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CHAPTER VIII

THE ADDUCTOR MUSCLE

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ANATOMY

The adductor muscle of the oyster is a massive organ that controls the opening and closing of the valves. It occupies a slightly asymmetrical position at the ventroposterior part of the body and is surrounded by the following internal organs: the visceral mass, pericardium, epibranchial chamber of the gills, and cloaca (fig. 72). The rectum adheres to the posterior side of the muscle. The protrusion of the visceral mass, containing the crystalline style sac and the lowermost part of the gonad, covers the anterior side of the muscle. A wedge-shaped visceral ganglion located inside the epibranchial chamber rests in a slight depression on the side of the muscle under the visceral protrusion. The ganglion can be exposed by cutting through the wall of the epibranchial chamber and lifting the tip of the visceral mass.

The adductor muscle of the monomyarian mollusks, i.e., those which have only one muscle (such as edible oysters, pearl oysters, scallops, and *Spondylus*), corresponds to the posterior adductor of other bivalves. The anterior adductor, present in larvae, disappears during metamorphosis shortly after the attachment of the larva.

Shortly after the metamorphosis of the larva the posterior adductor muscle develops into the most conspicuous and the heaviest organ of the oyster. In valves of *C. virginica* and in some other species of edible oysters the muscle scar where the adductor is attached to the shell is darkly pigmented. The shape and dimensions of this area are variable (see p. 30 ch. II).

The weight of the muscle of *C. virginica* accounts for 20 to 40 percent of the total weight of the tissues. After spawning, when other parts of the body are watery and poor in solids, the relative weight of the adductor increases. Examples of this condition, usually encountered after the discharge of a large number of sex cells and before the accumulation of the reserve materials (glycogen) in the connective tissue, are given in table 18. It may be deduced from these data that the weight of the adductor muscle is not affected by the changes in the chemical composition which take place in other organs. For further discussion of this problem the reader is referred to chapter XVII of this book.

The adductor is comprised of two distinct parts. About two-thirds of the total bulk of the muscle is translucent, oval-shaped, and slightly concave at

TABLE 18.—*Relative weight of the adductor muscle of six adult* C. virginica *(4 to 5 inches in height) during the spawning season (August) in Woods Hole, Mass. (fresh basis), 1951*

Oyster	Weight		Adductor muscle (total weight)
	Meat	Adductor muscle	
	Grams	*Grams*	*Percent*
Ripe male	17.8	3.5	19.7
Ripe male	15.0	3.7	21.8
Ripe female	18.7	4.4	23.5
Ripe female	6.5	2.1	32.3
Partially spawned female	6.5	2.3	35.3
Spawned out, sex undetermined	5.2	2.2	42.3

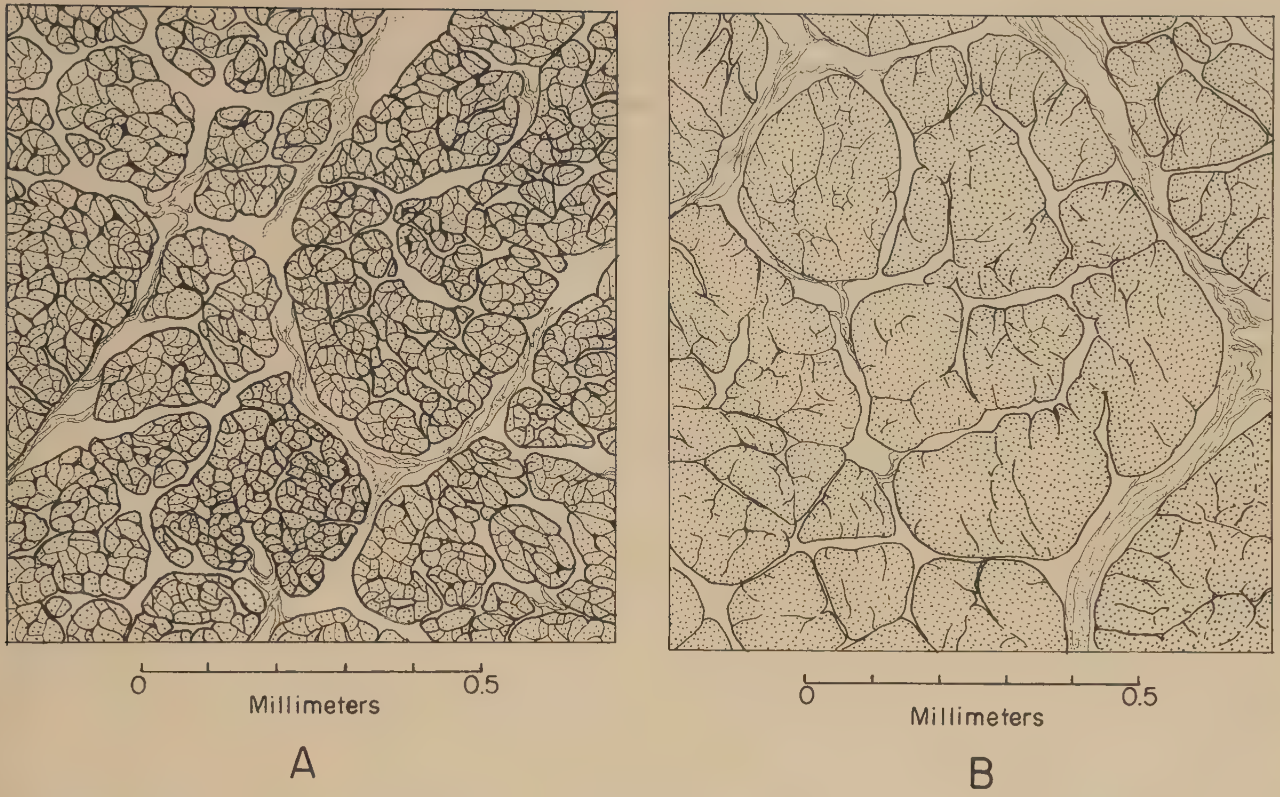

FIGURE 141.—Cross sections of the two portions of the adductor muscle of *C. virginica*. A—white or opaque part. B—translucent part. The muscle bands of the white part are more compact and are surrounded by tougher connective tissue than those of the translucent part (right). Bouin, with formalin hematoxylin-eosin.,

the dorsal side adjacent to the pericardium. This portion is frequently called the vitreous or dark part. The remainder is crescent-shaped and an opaque milky-white. The fibers of this part are tougher than those of the translucent portion; the difference shows clearly when the muscle is being cut or teased.

The fibers of the adductor muscle form dense bands surrounded by connective tissue. In a cross section examined under a low-power microscope (fig. 141) the bands appear as separate units packed more or less parallel to one another. This arrangement is less pronounced in the translucent part (fig. 141, right). The tissue that surrounds the muscle bands is better developed in the opaque section. A layer of connective tissue separates these two major parts of the adductor.

Connective tissue provides a framework for the muscle. Individual fibers do not run the full muscle distance between the two valves; they are anchored at one or both ends in the sheets of tissue which surround the bands. A very thin membrane, called endomysium, invests each muscle cell; the sheathing around the bands of cells is epimysium; the septa which radiate from the latter form perimysium.

The cross-sectional areas and the weight of the two portions vary in different specimens. It was reported by Hopkins (1930) that the ratio of weight of the translucent to the white part of the muscles of oysters growing near Beaufort, N.C., depends on ecological conditions. In the oysters found at the upper limit of their vertical distribution near the high-water level the ratio was 1.26, while in the oysters taken at a level 2.5 feet lower, where they were submerged during about three-quarters of the time, the ratio was 2.51.

The entire adductor muscle is well supplied with blood; wandering leucocytes are usually seen between the fibers and in the connective tissue. Both parts of the adductor muscle are abundantly supplied with nerves. The innervation of the muscle is discussed in Chapter XII.

MICROSCOPIC STRUCTURE

The muscle fibers of the two parts of the adductor differ in both size and structure. The white muscles are smooth and wide, while the dark (translucent) fibers are thinner and have a peculiar striation which has been described as oblique, double-oblique, helicoidal, and spiral. Some investigators (Kellogg, 1892; Orton, 1935; Hopkins, 1936) and authors of biology textbooks (Borradaile and Potts, 1961) refer to the translucent part as consisting of striated muscles.

Both types of fibers appear under the light microscope as long cylindrical cells, slightly thickened in the middle and tapering toward the ends. An oval-shaped nucleus with one or several nucleoli is near the surface, outside the contracting elements which make up the bulk of the cell. Clear homogenous cytoplasm (sarcoplasm) which can be seen under high magnification forms a very thin surface layer of the cell and around the nucleus. The major part of the cell is made of slender fibrils that differ in their orientation in the two types of muscle cells.

The principal structural elements appear in unstained, isolated fibers examined with phase contrast oil immersion lens under high magnification. Whole mounts can be made after pieces of muscle are macerated in 20 percent nitric acid and then placed in glycerol. Treatment with nitric acid apparently does not affect the visible structure of the fibers. Preparations should be made from fibers which have been taken from both a fully relaxed and a completely contracted adductor. The desirable state of relaxation is obtained by narcotizing the oyster in 5 to 10 percent magnesium sulfate solution for 48 hours; treating the mantle with a strong solution of hydrochloric acid causes long-lasting contraction. In opening the oyster, care should be exercised not to damage the visceral ganglion, since injury to this nerve center may cause relaxation of the adductor.

WHITE MUSCLE FIBERS

White muscle fibers isolated from a completely relaxed adductor of a fully narcotized *C. virginica* are from 2 to 3 mm. long and about 10 μ in diameter. The fibers are too short to stretch from one valve to the other and, with the exception of those attached to the shell, end in connective tissue. Occasionally they bifurcate but do not anastomose. The body of the fiber consists of many fibrils of variable length and a diameter of only a fraction of a micron. The fibrils are oriented parallel to the long axis of the cell and those close to the surface appear to be darker. The arrangement of the fibrils changes somewhat, depending on the state of contraction. Figure 142, A–D, represents four camera lucida drawings made of a white muscle fiber; (A) the fiber is in a completely relaxed state, (B) it is strongly contracted, (C) it is partially contracted, and (D) a noncontracted fiber is folded by the contraction of the surrounding fibers. All drawings were made from glycerin-mounted preparations examined with phase contrast lens. The difference between the relaxed and contracted fiber is primarily in the thickness of the fiber, which in B is about three times greater than in A. In both cases the orientation of fibrils is the same. In a partially contracted and slightly twisted fiber, C, some of the fibrils are at an angle to the long axis of the cell while others retain their original orientation. The fiber D, found in the same preparation with C, is folded but not contracted. Its surface layer of transparent cytoplasm was wider than in the others and the fibrils followed the zig-zag outlines of the fiber. Although the sample was isolated from a contracted adductor, only a few fibers were found in highly contracted state B. The fiber A was separated from a completely relaxed muscle.

DARK MUSCLE FIBERS

The fibers of the dark (translucent) part of the adductor are from 1 to 2 mm. long and in a relaxed state are about 5μ in diameter. When isolated in teased preparations, the fibers have a tendency to twist and coil. The connective tissue around them is less tenacious than in the white muscle, and the fibers can be separated easily by fine needles. As early as 1869 Schwalbe showed that the fast adductor muscle of *Ostrea* is composed of fibers which exhibit a clearly defined diamond lattice pattern. Marceau (1909) maintained that double obliquely striated muscles are widely distributed in the fast parts of the shell closing muscles of bivalves, and Anthony (1918) advanced a theory that oblique striations are a stage in the evolutionary development of transverse striation. The fact that true cross striation occurs in the muscles of *Pecten*, *Lima*, *Teredo*, *Spondylus*, and other bivalves leads to a widely accepted belief that the dark portion of the adductor muscle, also described by some authors as yellow, grey, or tinted (Kawaguti and Ikemoto, 1959), consists of cross striated fibers and that quick movements of these animals are brought about by their contraction.

From their study of the translucent fibers of the adductor of *C. angulata*, Hanson and Lowy (1961)

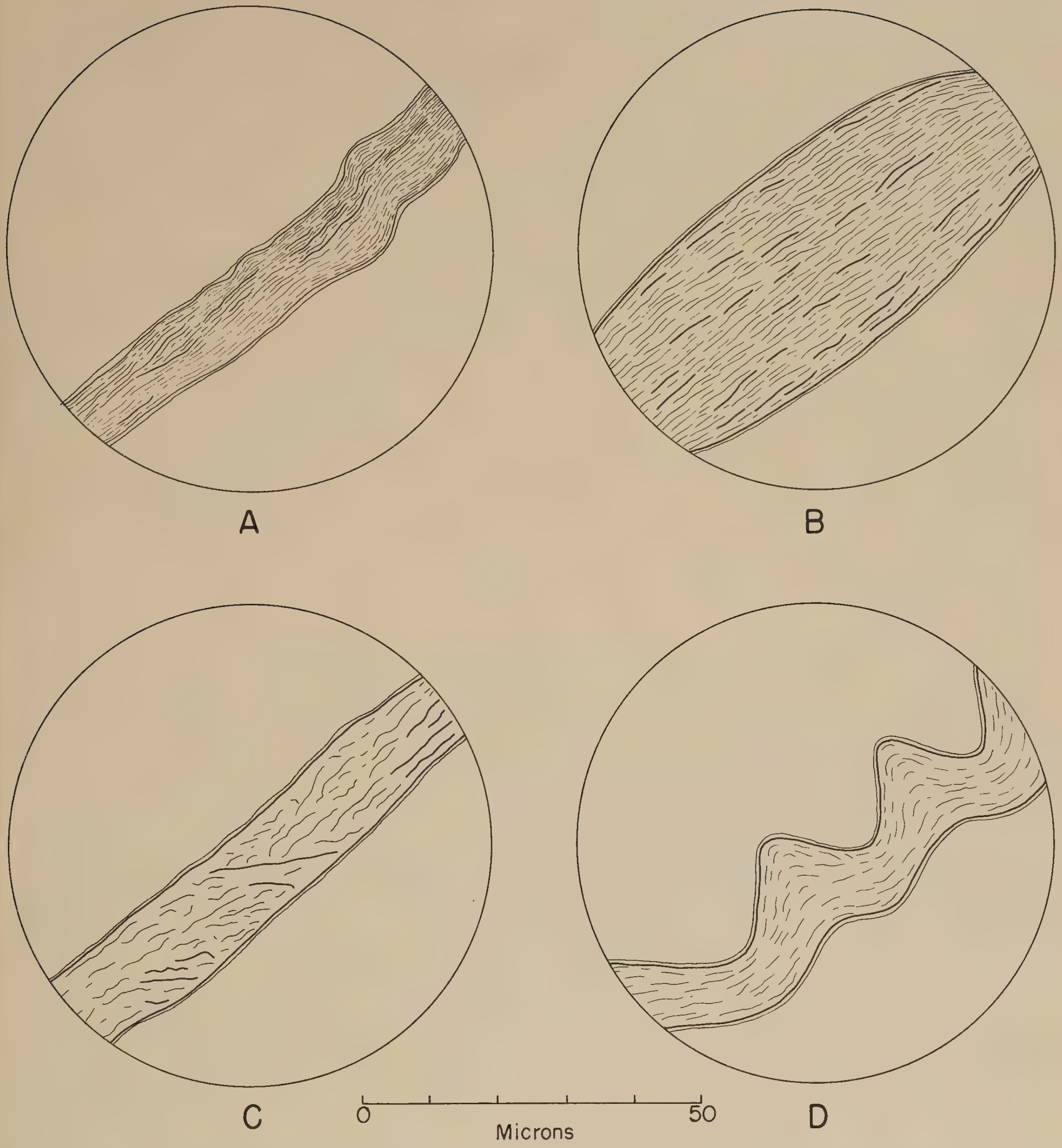

FIGURE 142.—Small pieces of four white fibers of the adductor of *C. virginica* seen under phase contrast lens. Whole mounts of a preparation teased after treatment with nitric acid. Glycerol. A—completely relaxed fiber from narcotized oyster; B—strongly contracted fiber; C—slightly contracted fiber; D—folded but not contracted fiber. Figures B and D are from one preparation of a highly contracted adductor.

concluded that the fibers of that part of the muscle differ from true cross striated muscles in that the bands (A and I) lie at about a 10-degree angle to the fiber axis and are arranged helically around the outer part of the fiber; this produces the double oblique striation visible in the light microscope. Hanson and Lowy's observations were based on electron microscopy, and the bands they refer to as A and I are not visible under the light microscope.

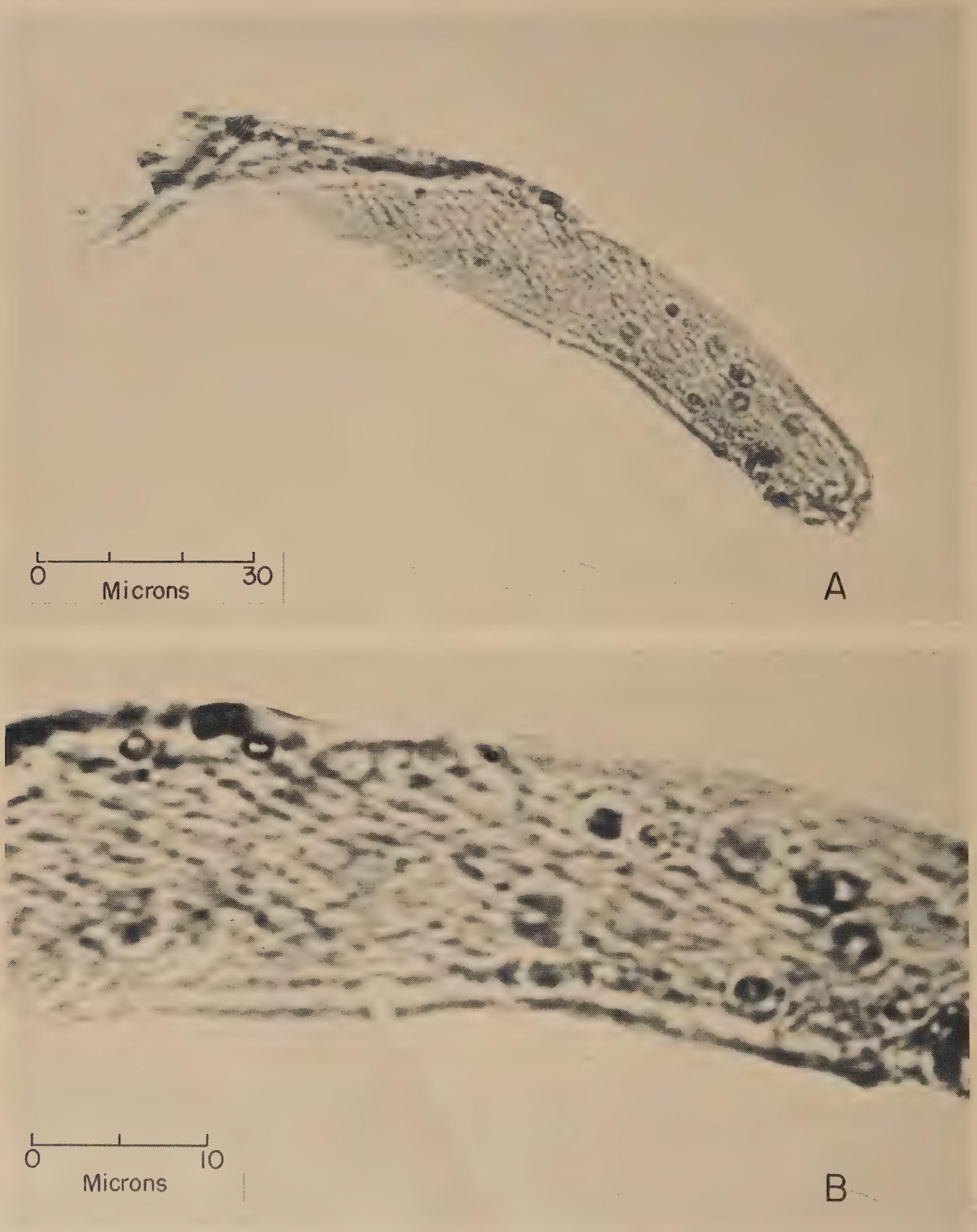

Figure 143.—Small piece of dark muscle fiber from the contracted adductor muscle of *C. virginica*. A—Whole mount in glycerol after nitric acid treatment. B—Small portion of the same negative magnified. Round globules are artifacts. Phase contrast lens.

Examination of relaxed fibers of *C. virginica* with phase contrast lenses shows the existence of a distinct diamond lattice pattern shown in figure 143. In the relaxed dark fibers this double oblique striation is absent and the fibrils are oriented parallel to the axis of the cell. My observations confirm the description made by Hanson and Lowy (1957), who found that in helical configuration of myofibrils of the "yellow" part of the adductors of oysters and *Ensis ensis* the angles between the helix and the axis of the fiber increased as the muscle relaxed. The so-called diamond lattice pattern of striation is not a permanent feature of the translucent fiber. It becomes visible in a contracted muscle and is usually confined to the cut ends of the fiber. This observation made by Bowden (1958) for *Ostrea edulis* and *C. angulata* is in accordance with my observations on *C. virginica.*

Considerable advance in the understanding of fine structure of bivalve muscle cells was made by Philpott, Kahlbrock, and Szent-Györgyi (1960), in the work on *C. virginica, Mya arenaria, Mercenaria mercenaria,* and *Spisula solidissima.* Similar studies of *C. angulata* were made by Hanson and Lowy (1961).

With respect to the ultrastructure of the fibers of the adductor muscles of these species, the results of the two investigations are in agreement although they present different theories of the so-called catch mechanism of the adductor, which is discussed later. In both parts of the muscle the fibrils consist of two types of filamentous structures that can be clearly seen on the electron micrograph of the transverse section of the fibril (fig. 144). The thick filaments form the largest part of the fibril; their diameter varies from 250 to 1,500 Å. The thin filaments which occupy the space around the thick ones are about 50 Å. in diameter. The thick filaments have the 145 Å. periodicity associated with paramyosin. The authors surmise that actomyosin is localized in the thin filaments. Hanson and Lowy (1961), in confirming the presence of two kinds of filaments in the fibrils of *C. angulata,* assume that the thinner filaments contain mainly actin. Accord-

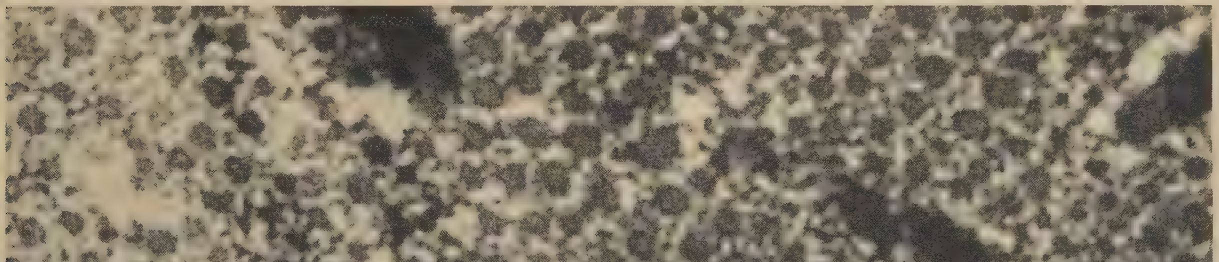

FIGURE 144.—Electron micrograph of a small portion of a muscle fiber of the translucent part of the adductor of *C. virginica.* Courtesy of Philpott and Szent-Györgyi.

ing to their interpretation of the electron micrographs which accompany their paper, both types of filaments are relatively short in comparison with the length of the fiber; they lie parallel to the fiber axis and are grouped with separate arrays which alternate with each other and appear to be cross-linked by means of transverse projections which belong to the thick filaments. The existence of the projections does not seem to be firmly established, and their connections with the two types of filaments require corroboration. It is obvious from the electron micrographs published by Philpott, Kahlbrock, and Szent-Györgyi, (1960) that filaments are randomly distributed throughout the cross-sectioned area of the fibril.

In the relaxed state the muscle cells are stretched and on longitudinal sections of either part of the adductor appear to be arranged in parallel lines separated in places by connective tissue (fig. 145).

A contracted adductor muscle is strikingly different in appearance from one which is relaxed. Most of the muscle fibers are folded and the entire organ has a herringbone appearance (fig. 146). The uniform thickness of the folded fibers indicates that their actual length is not shortened by the contraction; the fibers are compressed to occupy a shorter distance between the valves. Folding implies the existence of a force that acts parallel to the longitudinal axis of the fibers. The question arises as to the nature of the force that produces this effect. In an attempt to answer this question I examined a series of sections of muscles preserved at various degrees of contraction. Oysters were stimulated to close their valves and were preserved in that state by using a strong and rapidly acting fixative applied through an opening cut in a portion of the shell. In such preparations contracted muscle fibers were found only in the area near the attachment to the valves. In the two photomicrographs (fig. 147) the contracted fibers, nearest to the valve (left side), are short, thick, and deeply stained with eosin. The fibers to the right in the same preparation are narrow and folded.

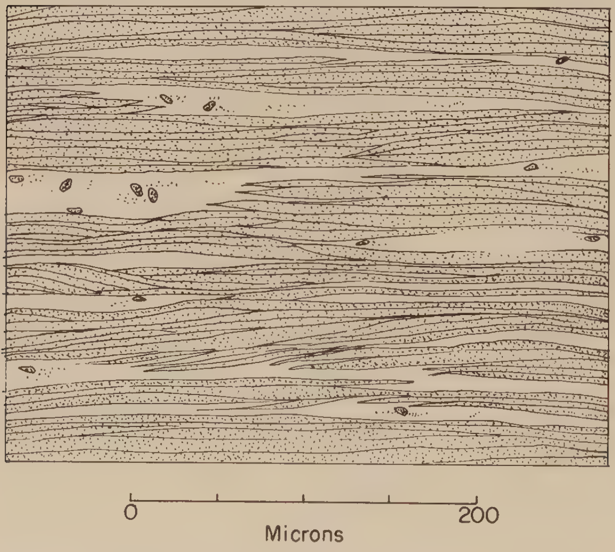

FIGURE 145.—Longitudinal section through a completely relaxed translucent part of the adductor muscle. Bouin, hematoxylin-eosin.

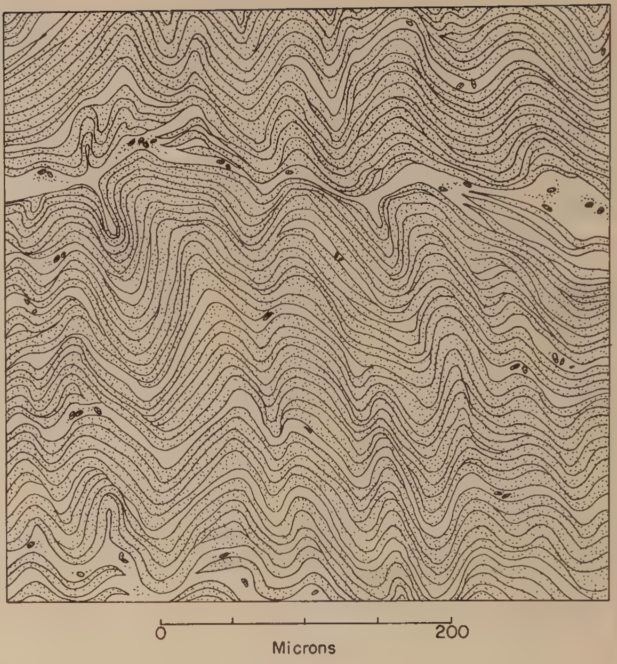

FIGURE 146.—Longitudinal section of the white part of the adductor muscle of *C. virginica* preserved in Bouin with formalin solution. Muscle is in a highly contracted state. Hematoxylin-eosin. Camera lucida drawing.

In a partially closed oyster the contracted fibers may be scattered between the folded fibers throughout the entire cross-sectional area. This, condition, shown in figure 148, is drawn from preparations preserved in osmic acid and stained with iron hematoxylin. The contracted fibers appear as isolated dark bodies scattered throughout the moderately folded fibers. It may be deduced from the histological picture that only a small number of muscle fibers are in a contracted state. In order to explain the folding of the noncontracted portion of the adductor it is necessary to assume that a rigidity develops in the contracted fibers in two places—near their contact

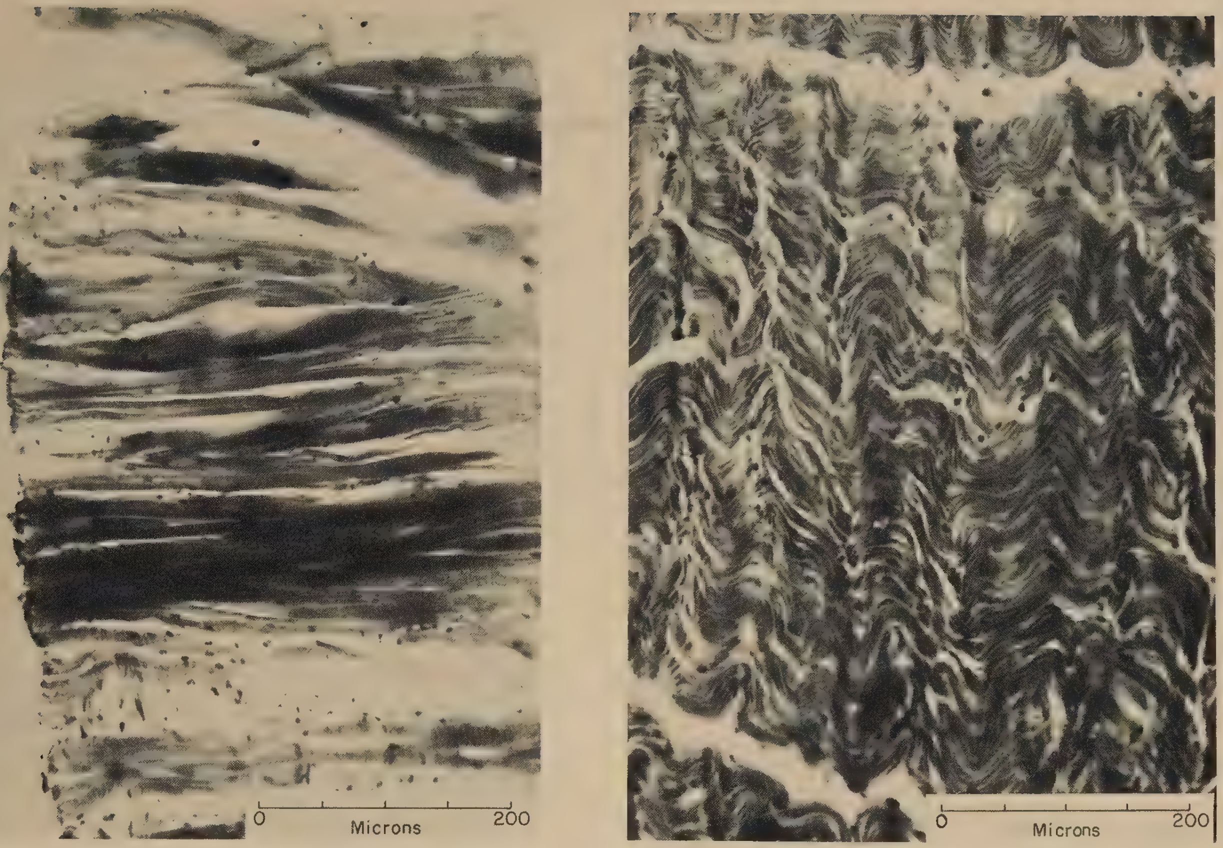

FIGURE 147.—Two photomicrographs of a longitudinal section of the translucent part of the adductor muscle near the valve (the left side of the photograph). The muscle was preserved in a contracted state in Bouin with formalin solution. Note the thick, short contracted fibers on the left and the beginning of folding at the right edge of it. Contracted fibers are deeply stained with eosin. The photomicrograph on the right shows folded fibers a short distance away from the area of the same section shown at left.

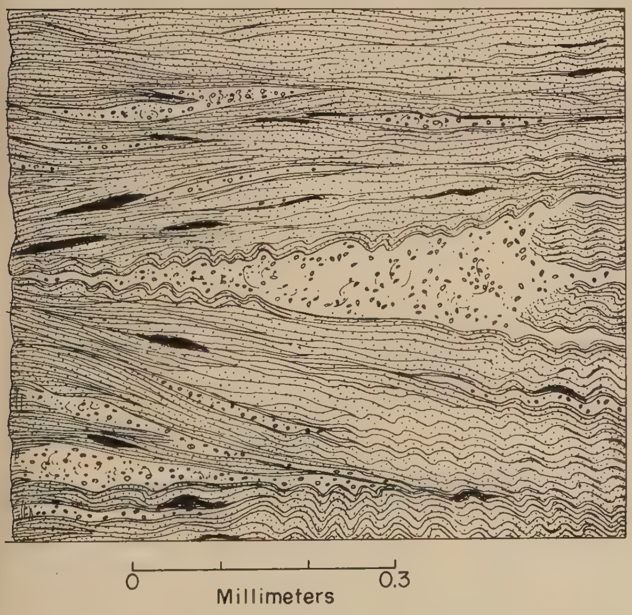

FIGURE 148.—Longitudinal section of partially contracted translucent portion of the adductor. Contracted fibers appear as black spindles. Osmium fixation. Iron hematoxylin.

with the folded fibers and at their anchorage in the connective tissue. Under this condition the contracted portions will bring the valves together and compress the noncontracted fibers into folds. This gives the oyster a considerable degree of flexibility in controlling the degree of opening of the valves.

Observations by Bandmann and Reichel (1955) on *Pinna nobilis* deal with similar conditions. In the smooth muscle of this mollusk plastic lengthening is combined with an orientation of the fiber structure without any changes of its elastic properties. The reverse process (disorientation) takes place during contraction, which is accompanied by an increase in dynamic stiffness. The authors attribute plastic and contractile length alterations to two different mechanisms: change in orientation and change in molecular shape within the contractile elements.

No observations have been made in the living oyster of the contractions of small bundles of fibers that run parallel to the surface of the valves.

These fibers, which are at right angles to the main fibers extending from one valve to the other, are found near the attachment of the adductor to the valves (fig. 149). Their position suggests that they act as braces by bringing together and tightening the principal bundles.

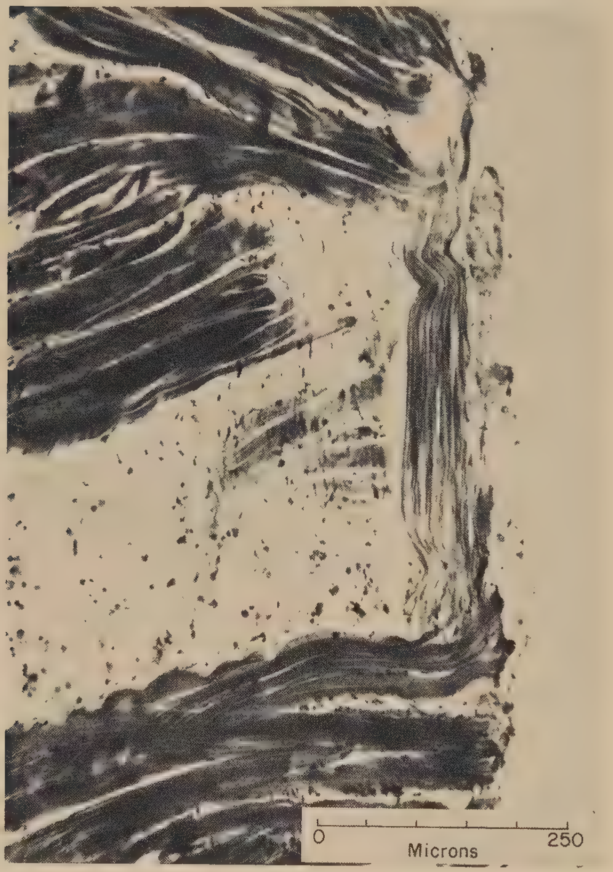

FIGURE 149.—Longitudinal section of a piece of partially relaxed muscle near the attachment to the valve (right side). Note band of muscles at right angle to the main fibers. Kahle, hematoxylin-eosin.

ATTACHMENT TO SHELL

The adductor muscle of *C. virginica* is fastened so strongly to the shell that when the valves are forced apart the muscle breaks in the middle instead of tearing from the shell. The adhesion sometimes withstands a pulling force of 10 kg. (22 pounds). On the other hand, the connection between the muscle and the shell can be weakened or completely destroyed by applying heat to the shell over the area of the muscle scar. This connection is smooth and glossy.

Brück (1914) found that in the shells of *Anodonta* and *Cyclas* the muscles are fastened by means of a specialized layer of cells which he called holding or adhesive epithelium ("haft epithelium").

Hubendick (1958) used both electron and light microscopy to demonstrate the presence of adhesive epithelium in the areas of attachment of the muscles of the fresh-water snail *Acroloxus lacustris* (Maxwell). The surface of the cells has a dense brush border of minute microvilli which are transversed by very thin cytoplasmic fibrils originating in the base of the cell. The epithelial cells are fastened to the underlying connective tissue by the evaginations which extend into the base of the cells. Since the muscles used by Hubendick were fixed in osmic acid, which resulted in their detachment from the shell, the electron micrographs published in his paper do not show the actual connection between the microvilli and shell material. The shell surface over the area of the attachment has, however, small depressions into which fit the tops of the microvilli. It is, therefore, likely that in *Acroloxus* the adhesion of the muscle is accomplished in this manner.

The holding epithelium of *C. virginica* can be seen on transverse sections of decalcified shell and muscle preparations. Individual cell boundaries are indistinct, but the position of each cell is clearly marked by a large round nucleus (fig. 150). Fine strands resembling those described by Hubendick originate in the base of the cells and terminate at their surfaces. They are not visible at low power but can be seen under oil immersion. The holding epithelium of the oyster is a modification of the surface epithelium of the mantle; the transition from one type to another can be seen in the areas adjacent to the muscle attachment (fig. 151). The holding epithelium of *C. virginica* secretes an organic film of about 2 μ in thickness that consists of adhesive material by which the muscle fibers are attached to the shell. The chemical nature of this film was not determined, but staining properties suggested the presence of collagen. Since it is known that under proper conditions collagen is digested by collagenase, I made a series of experiments at Woods Hole to determine the effect of this enzyme on the attachment of muscles. Small amounts of phosphate buffer solution (pH 8.4) containing 1 mg. of collagenase per ml. were injected into adductor muscles through holes

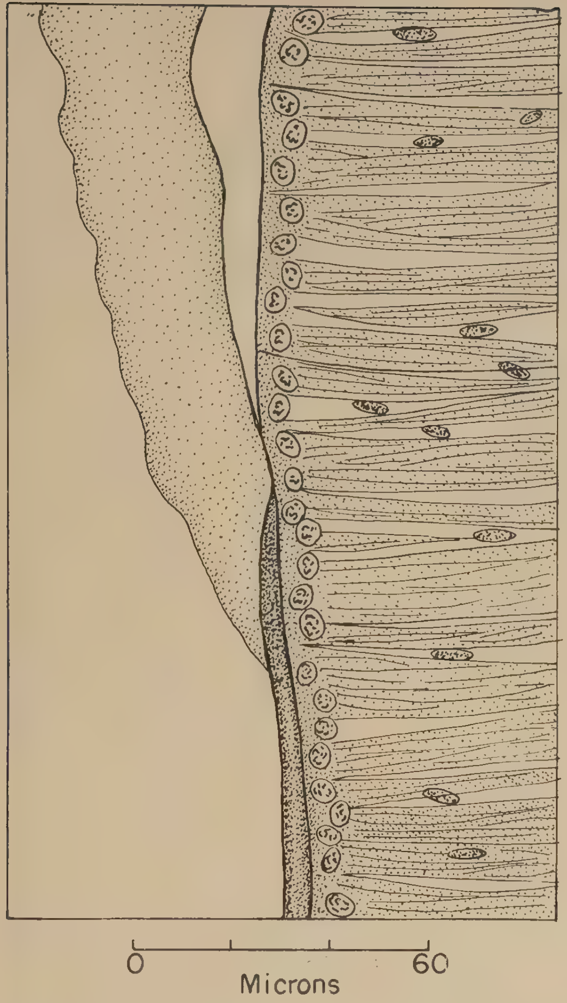

FIGURE 150.—Longitudinal section of the adductor muscle of *C. virginica* where the muscle fibers are attached to the shell. Note the holding epithelium and the cement layer which in the upper part of the illustration is separated from the epithelium and turned over. The decalcified shell is out of the field of view. The cement film is partially detached at the upper half of the preparation and twisted exposing its surface facing the shell; the width of the upper part of the film corresponds to the thickness of the section. Kahle. Hematoxylin-eosin.

drilled in the valves. In another set of experiments the muscles of oysters with the shells attached to them were immersed in the solution of collagenase and were kept at a temperature of 24° to 25° C. for 24 to 48 hours. Solutions of trypsin and phosphate buffer alone, without collagenase, were used for control experiments. In all cases the muscles treated with collagenase became detached within 36 hours. In the controls they remained attached to the shells (fig. 152).

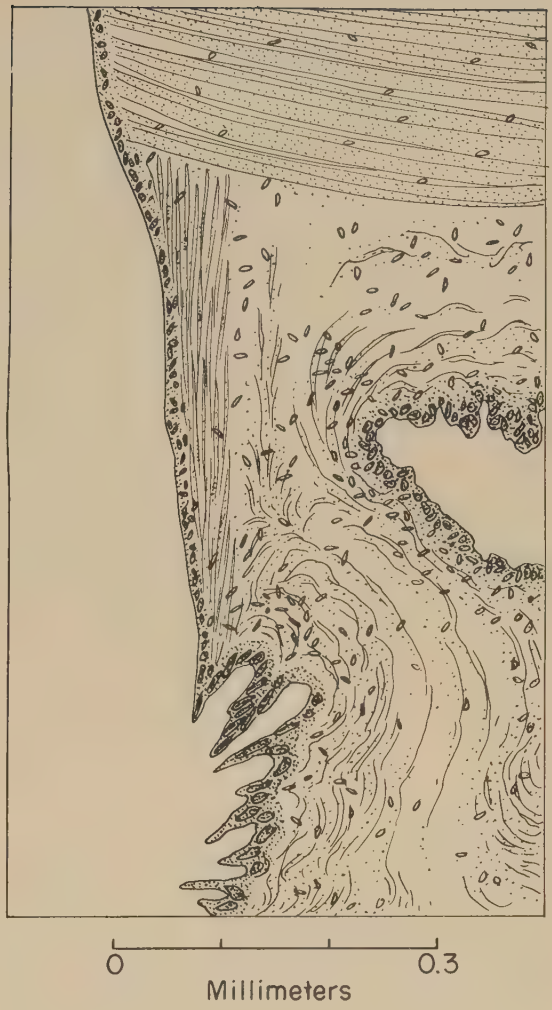

FIGURE 151.—Cross section of the visceral mass of *C. virginica* near the adductor muscle. Notice the gradual change of typical mantle epithelium (left side) into holding epithelium covering the adductor muscle. The shell is not shown. Kahle, hematoxylin-eosin.

CHEMICAL COMPOSITION OF THE ADDUCTOR MUSCLE

The chemistry of the adductor muscle of oysters has received less attention than that of the muscles of clams, scallops, and sea mussels. Probably the differences in the chemical composition of the muscles of various marine lamellibranchs are not of fundamental nature, although the proportion of various components may vary greatly between the species and even within mollusks of the same species living in different environments. Older reviews dealing with the comparative physiology of the adductor muscle make no distinction between the various groups of mollusks and combine the data under the general and nonscientific designation of "shellfish" (Katz, 1896; Riesser, 1936). Gross analysis of the adductor muscle of the oyster (*O. imbricata*) (Grimpe and Hoffmann in: Tabulae Biologicae, 1926) shows the following composition: water 66.58 percent; protein 11.38 percent; fat 4.8 percent; and ash 1.1 percent.

INORGANIC SALTS

Studies of the content of the metallic salts in the body of oysters and other bivalves were made by many investigators interested in the problem of osmotic regulation in marine invertebrates. Observations on European oysters, presumably *O. edulis*, made by Krogh (1938) are of particular significance. He found that in the oysters living in waters of high salinity (35°/oo) in France the concentrations of chlorine, sodium, and potassium expressed on the basis of tissue water, were as follows: chlorine 256 mM/kg.[6]; sodium 265 mM/kg.; potassium 46 mM/kg. The next day the oysters were placed in water of lowered salinity (25°/oo) in Limfjord, Denmark, and individual samples were taken at intervals of 1 to 2 days. The results, though somewhat irregular owing to individual variations, showed a decrease in chlorine (221 to 138 mM/kg.) and in sodium (258 to 139 mM/kg.). The potassium increased from 46 to 98 mM/kg.

The mean values for the concentrations of some elements in the adductor muscle of the Australian oyster, *Crassostrea* (*Saxostrea*) *commercialis*, were found to be as follows (Humphrey, 1946):

	Percent Mg.
Potassium	381. 7 ±18. 9
Sodium	327. 9 ±13. 0
Calcium	45. 76± 3. 28
Magnesium	79. 93± 3. 03
Chlorine	733. 4 ±17. 3

In this case sodium and potassium were present in almost equal amounts (Na:K=0.98) while in *O. edulis* potassium was present in much smaller concentrations and the Na:K ratio varied from 1.6 to 5.8. The concentrations of calcium and magnesium in the whole adductor muscle of *C. commercialis* were found to be 1.1 and 1.5 x 10^2 M, respectively. Both elements are uniformly distributed between the two parts of the muscle (Humphrey, 1949).

ORGANIC COMPONENTS

Glycogen

Bivalve mollusks accumulate considerable quantities of glycogen in their tissues, including the muscles. This reserve material is deposited primarily in the connective tissue of the body parenchym and in the mantle and in smaller quantities is found in the gills and adductor muscles. Analyses made in the Bureau's shellfish laboratory show that on a percentage basis the adductor muscle stores smaller quantities of glycogen than do the gills or visceral mass (table 19).

TABLE 19.—*Solids, water, and glycogen content of the adductor muscles, gills, and remainder of the bodies of* 15 C. virginica *of good quality from the vicinity of Charles Island, Long Island Sound*

[Average percentages of fresh substance, 1934, 1935]

Item	Mantle	Body	Gills	Adductor muscle
November 30:				
Water		73.24	80.20	78.57
Total solids		26.76	19.80	21.43
Glycogen		7.96	4.68	1.69
January 3:				
Water	78.60	74.0	88.52	79.04
Total solids	21.40	26.0	11.48	20.96
Glycogen	3.37	3.96	1.53	1.40

Samples consisted exclusively of adult Long Island Sound oysters of good commercial quality and high content of solids; they were analysed within a few hours after removal from the bottom.

In the Japanese species, *O. circumpicta*, the percentage of glycogen in the two parts of the

FIGURE 152.—Effect of collagenase on the attachment of the muscle of *C. virginica*. Upper row: control—trypsin injected through the hole in the right valve (on left) has no effect on the attachment of the muscle. Lower row: part of the adductor is detached from the right valve after an injection of collagenase. The detached part is seen on the left valve (right side). Twenty-four hours after injection, 24° to 25° C. Left valves of each oyster are on right.

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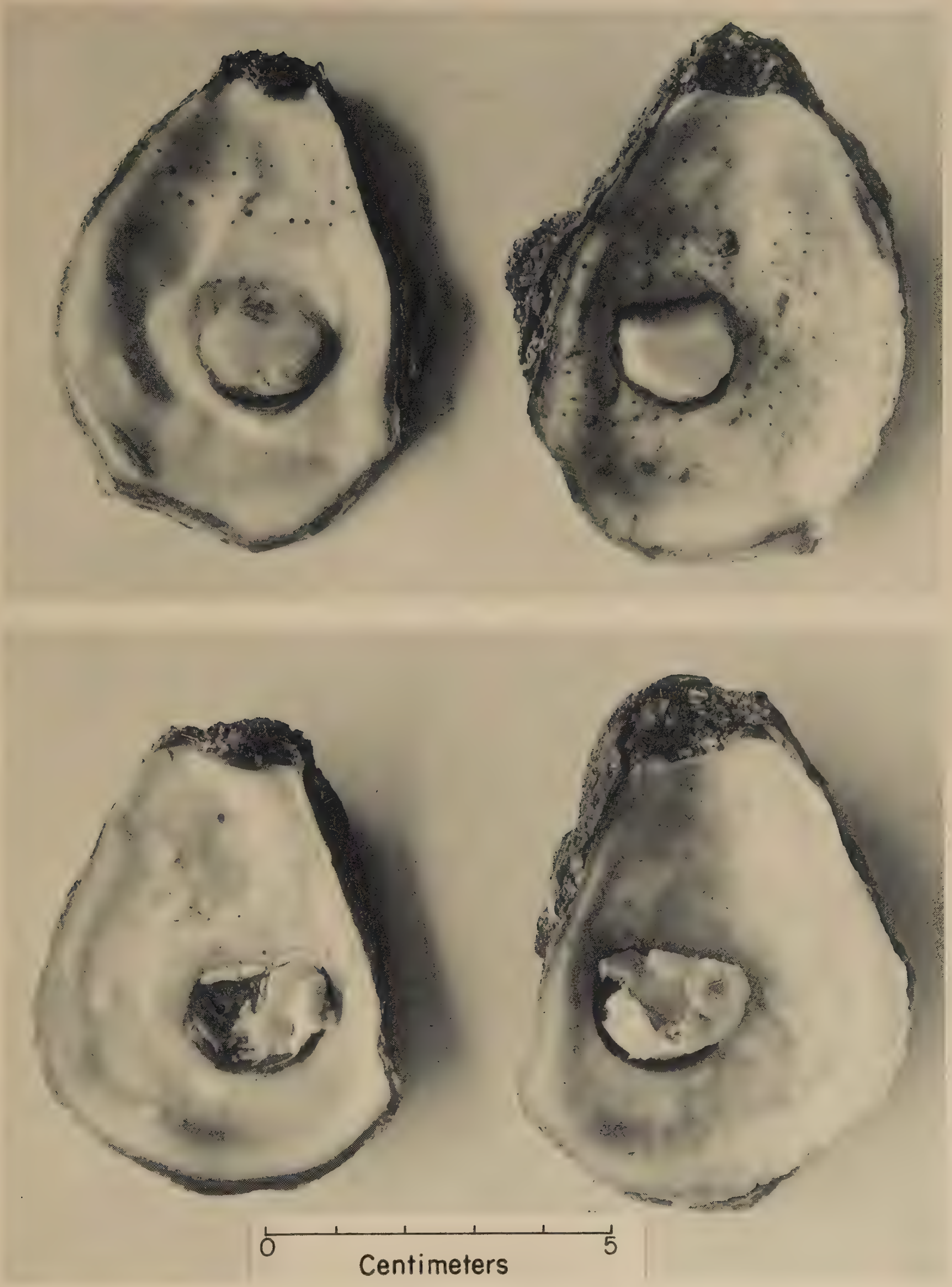
[6] Values given in millimoles per kilogram of water.


0
5
Centimeters

adductor has been calculated as follows (Kobayashi, 1929):

Translucent portion—	
October	1.12 percent.
November	1.0 percent.
White portion—	
October	1.43 percent.
November	1.29 percent.

The figures are not essentially different from those for *C. virginica*. The questions of how much of the glycogen in the adductor muscle is part of the muscular mechanism and how much of it is stored have not been answered with certainty.

Proteins

According to the data quoted from Tabulae Biologicae (1926), the fresh adductor muscle of *O. imbricata* contains 11.38 percent protein and 4.8 percent fat. No published data are available for the protein content of the muscle of *C. virginica*. It may be assumed that in this species the protein content is not essentially different from that usually found in plain muscles in which it forms from 14 to 18 percent (Evans, 1926).

The contractile mechanism of the adductor muscle of bivalves has the same structural elements as are found in vertebrate muscles: myosin (Florkin and Duchâteau, 1942), actin, and adenosinetriphosphate (ATP). The actin and myosin extracted from muscles of *O. edulis*, *Mytilus edulis*, and *Pinna nobilis* (Lajtha, 1948) have solubility relationships similar to those of the corresponding substances of rabbit muscle (Szent-Györgyi, 1951). The myosin is soluble in distilled water, insoluble in dilute potassium chloride solution (0.002–0.08 M), and again soluble in 0.1 M potassium chloride and higher. It is also soluble in the 0.1 M and stronger solutions of chloride and magnesium chloride. Myosin and actin can be precipitated at isoelectric points of 5.2 and 4.7. They both show double refraction which disappears in dilution or at higher concentration (0.4 M potassium chloride for myosin). Actin has a higher double refraction than myosin. It also has the peculiar property of undergoing reversible change from the globular to the fibrous state and vice versa, depending on the pH and ionic concentration of the medium.

Besides actin and myosin the adductor muscle contains another protein called paramyosin, which differs in solubility and X-ray diffraction from myosin (DeRobertis, Nowinski, and Saez, 1954). Paramyosin was first detected in the adductor muscle of the clam (*Mercenaria* (*Venus*) *mercenaria*) by using electron stains (Hall, Jakus, and Schmitt, 1945). Preparations of muscle fibrillae treated with phosphotungstic acid reveal a periodic structure of alternate bands that show affinity for the stain. The distance between the bands averages 145 Å. At the same time there is a larger period of 720 Å. which is repeated every five spaces of the smaller period (145 × 5). It was concluded by Hall, Jakus, and Schmitt (1945) that the fibrillae of this type consist of paramyosin. Its content in various bivalves varies but is quite high in *Mytilus edulis* in which, according to Lajtha (1948), it exceeds the content of myosin. Paramyosin of the adductor muscle of *C. virginica* was separated from actomyosin by precipitation with three volumes of ethanol at room temperature (Philpott, Kahlbrock, and Szent-Györgyi, 1960) and resuspension of the precipitate in 0.6 M potassium chloride at pH 7.4, which was then dialyzed against the same solution. By such treatment the paramyosin passed into solution and the actomyosin remained precipitated. The yield of paramyosin extracted in percent of total protein was 22 percent in the opaque part, 16 percent in the translucent part. On the basis of biochemical studies the authors suggest that paramyosin is localized in the thick filaments, while the thin filaments consist of actomyosin.

Paramyosin is not found in vertebrate muscles but is the principal protein in many invertebrates (Engström and Finean, 1958). Although its particular role in muscular contraction has not been determined with certainty, it appears probable that this protein is responsible for the maintenance of the tonus of the adductor muscles.

PHYSIOLOGY OF THE ADDUCTOR MUSCLE

The zoologists of the middle 19th century were aware of the difference in the function of the two parts of the adductors of bivalves. They regarded the white part as a bunch of elastic bands which counteracted the pulling force of the valve ligament and the translucent part as an ordinary muscle which brought the valves together (Bronn, 1862, p. 359). Coutance (1878) and Jhering (quoted from Marceau, 1904a) and later Jolyet and Sellier (1899) maintained that the translucent part of the adductor muscle of *Pecten maximus* consists of striated anastomosing fibers whose exclusive function is to close the valves; they observed that the white part of the adductor contracts very slowly and can remain in a contracted state for a long time. Marceau (1904a, 1904b) confirmed these results by a series of experiments. He cut off either white or translucent portions and found that in *O. edulis* the rapid closing of the valves is accomplished by the contraction of the translucent part of the muscle while the elasticity and tonus of the white part counteract the pulling force of the ligament. Useful reviews of many investigations dealing with the muscle physiology of bivalves and other invertebrates are found in the papers of Ritchie (1928), Jordan (1938), Evans (1926), and others.

It is a well-established fact that the two parts of the adductor muscle contract at different speeds. In scallops the isolated striated (translucent) portion contracts in about 100 microseconds (μ sec.); its relaxation time is about 0.1 second (sec.) (Bayliss, Boyland, and Ritchie, 1930). In the slow part of the adductor the contraction time varies from 500 μ sec. to 2.5 sec. and the relaxation time is from 10 to 45 sec. The contraction of the adductor muscle of oysters is always several times faster than its relaxation, the ratio varying according to the type of muscular reaction. Marceau (1909) published a number of tracings of the spontaneous movements of the valves of *O. edulis* in which only the white (slow) part of the muscle was left. The time of relaxation was from 15 minutes to 1 hour.

In many bivalves the adductor muscle can remain contracted, keeping the valves closed tightly, for a long time. This behavior varies, however, in different species. For instance, common scallops of the American and European coastal waters, *Astropecten irradians* and *Chlamys opercularis*, close their valves for only a short time. Soon after being taken out of water they gape, lose shell liquor, and perish. My observations on pearl oysters of the Hawaiian Islands and Panama (*Pinctada galtsoffi*, *P. mazatlanica*), show that shortly after being taken out of water their shells gape and the muscle fails to contract. These species cannot be transported over long distances unless they are kept in frequently renewed water all the time. On the other hand, the bivalves in which the adductor muscle remains contracted for a long time can survive long exposure and can be shipped alive over great distances.

Oysters living within the tidal range on flats thrive in this situation because they can keep their valves closed during the time of exposure. It is obvious that this ability provides a great survival value for those sedentary animals that can withdraw within their heavy shells to avoid desiccation and remain protected against unfavorable conditions or attacks of predators.

The ability of bivalve muscles to keep the shells closed is frequently called a "catch" or locking mechanism. The idea originated from observations made by Uexküll (1912) on the scallop; if a piece of wood is pushed between the valves the adductor contracts with such force that the edges of the shells may be splintered. The wooden wedge is held as firmly as if it were in a vise and can be removed only by twisting and pulling. The valves, however, remain motionless, and the muscle that holds them in their position shows no elasticity. The muscular fibers seem to be frozen solid. The shell cannot be opened, but if the valves are pressed on both sides they may be brought nearer together and remain fixed in their new position. This ability Uexküll called "Sperrung", which in English means "locking." Bayliss (1924) interpreted Uexküll's expression using the word "catch," probably influenced by Grützner's (1904) suggestion that the muscle fibers of the bivalve adductor must somehow be "hooked up" by a mechanical arrangement similar to a ratchet consisting of two pieces with teeth facing each other. In his proposal the upper piece could be pushed only in one direction, shortening the total length of the model, and the upper teeth could not move back unless the two pieces were separated from each other by the depth of the teeth. There is nothing in the structure of the muscle fiber which even remotely suggests the existence of such a mechanism. The expression "catch mechanism" implies some mechanical device and is, therefore, misleading. It has been used, however, for such a long time that the literary meaning of the words has been lost and the term simply refers to the continuous state of contraction of the closing muscle of bivalves.

Several theories have been proposed to explain the locking or catch mechanism of the adductor muscles. Some investigators assumed that the muscle twitch (i.e., the contraction in response to single brief stimulus) is common to all muscles and the difference between the behavior of the adductors of bivalves and of the muscles of other types is due to the differences in time scale and the condition of stimulation. It was claimed, (Ritchie, 1928, p. 86), although not proved, that tonus of the adductor muscle is maintained by tetanic contraction. Another view (Winton, 1930), which is more in harmony with the biochemical data, explained the locking mechanism as a result of physical changes during contraction, particularly the alteration in viscosity of muscle proteins. Experiments with byssus retractor of *Mytilus* showed that after stimulation by direct current the viscosity of the muscle was raised and remained high for about 2 hours. No such effect was obtained if alternating current was used. These observations suggest that viscosity changes are involved in the contractions of the adductor of bivalves.

The difference between the white and the translucent parts of the adductor muscle may be primarily of a quantitative character. This suggestion was made by Shukow (1936), who found that in *Anodonta* and *Unio* the two parts of locking muscles actively participate in single, spontaneous contractions and in the maintenance of tonus. Shukow's observations indicate the inadequacy of the theory that makes the maintenance of the tonus the exclusive function of the white fibers.

Studies of the electric phenomena in the smooth adductor muscles of lamellibranchs (*Mytilus*, *Modiolus*, and smooth part of *Chlamys*) lead Lowy 1953, 1955) to conclude that the hypothesis of "catch mechanism" is unnecessary because, according to his observations, the tonus in the intact muscles of these mollusks is due to a shifting pattern of tetanic stimuli controlled by the nervous system, bringing it in line with the tonus in other muscles. Since action potentials were observed in muscles which were isolated from the ganglia, Lowy suggested that they may be of myogenic nature. The question of whether the tonic activity of lamellibranch muscles is neurogenic or myogenic remains open. Lowy makes an interesting statement that "lamellibranch muscles maintain a certain level of tension all the time due to the activity of a peripheral automatic system, which works by successive activation of limited areas."[7] This conforms with the histological observations described above which show that in an intact adductor muscle of the oyster preserved in a contracted state only certain muscle bands are in a true contracted state while others are folded. Lowy concludes that further studies are needed before it is decided whether lamellibranch muscles are directly innervated by excitatory and inhibitory nerves or are acted on indirectly via a peripheral ganglionic plexus. The existence of inhibitory axons in *Pecten* was demonstrated by Benson, Hays, and Lewis, (1942), who found that the relaxation of the adductor of the scallop was considerably accelerated by stimulating certain nerve bands going to the msucle. This is in accord with the evidence presented by Barnes (1955) for the adductor muscles of *Anodonta*. His work implies that the adductor of *Anodonta* is innervated by three types of nerves: one group of motor fibers supplies the striated muscles and produces phasic contractions which may summate and produce tetanus; another group of activating fibers supplies the unstriated muscles and produces increased tonus; the third group consists of inhibitory fibers which decrease the tonus. Barnes points out that the nervous mechanism controlling the adductor activity in *Mytilus* may be the same as in *Anodonta*. *Mytilus* is capable of both phasic and tonic contractions, but there is no obvious differentiation of the muscle into two parts. It must be accepted, therefore, either that all muscle fibers are capable of exhibiting both types of contraction or that the two types of fibers are present but completely interspersed.

Electrical activities associated with the contraction of the adductor muscle of the oyster have not been studied enough to warrant an evaluation of their role in the locking mechanism of these mollusks. An attempt to solve the paradox of the catch muscle mechanism was made recently by Johnson, Kahn, and Szent-Györgyi (1959) and is based on the study of the property of paramyosin. The solubility of this protein was found to be critically dependent upon the pH and ionic strength of the medium. Similar dependence was shown in the glycerinated fibers of the anterior byssus retractor of *M. edulis*. The fibers were stretched, and the tension thus developed was measured. To reduce the effect of actomyosin, 10^{-4} M Salygran and 10^{-2} M pyrophosphate were added to the medium. Stiffness of the fibers was measured at various values of pH. Below pH 6.5 and at low ionic strength of 0.07 M potassium chloride the fibers were relatively stiff. This is a range in which paramyosin crystallizes out of solution. At higher pH values the fibers were relatively plastic. The authors think that parallel with the actomyosin system which produces initial tension of the adductor there is a second, or paramyosin system, capable of maintaining the tension developed by the first one by crystallization of the paramyosin component caused by pH shift within the muscle. The theory was tested by Hayashi, Rosenbluth, and Lamont, (1959) on the muscle extracts of *Mercenaria* (*Venus*) *mercenaria* and *Spisula solidissima*. The results of these experiments tend to support the hypothesis that crystallization of paramyosin effectively freezes the adductor muscle at any state of contraction.

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[7] —Underscoring is mine. P. S. G.

In two papers dealing with the fine structure of the small fibers of the oyster (*C. angulata*) and other bivalves, Hanson and Lowy (1959, 1961) have proposed two possible explanations of the mechanism by which the closing muscles of mollusks maintain tension "very economically," i.e., without using much energy. According to their view, based on examination of electron micrographs of muscle, the thick filaments of the fibril (see p. 157 and fig. 144) are discontinuous and do not contract; they slide as the muscle shortens the relative portions of the thick and thin filaments. The tension is maintained by cross links between the two types of filaments. According to their view the alternative hypothesis, which supposes that tension is maintained by change in the physical state of the protein within a paramyosin system, is difficult to reconcile with their observations. The sliding or so-called interdigitatory model of the contractile structure is based primarily on the studies of striated muscle (Huxley, 1960), and the extension of the theory to nonstriated muscles of bivalves is very attractive. It is impossible, however, to state at present which of the two theories interprets correctly the catch mechanism. Further experimental studies are needed to solve the puzzle which for a century has baffled the biologist.

In spite of the substantial advance of biochemical investigations, the problem of the locking mechanism requires further study. So far no evidence has been presented to show that the shift in the pH needed for the crystallization of paramyosin actually takes place in the whole living muscle of a bivalve. It seems that the solution to the locking paradox should consider the problem in its entirety, by taking into account all the biochemical and biophysical processes which accompany the prolonged tonus of the adductor muscle.

Chemical changes during muscular activity

Chemical changes occurring during the contraction and relaxation of the muscle are extraordinarily complex. The reader interested in this problem should consult the textbooks of general physiology (Scheer, 1948), biochemistry (Needham, 1932; Baldwin, 1957), and particularly the comprehensive reviews of more recent works given by Szent-Györgyi (1951) and Weber (1958). Most of the work on the chemistry of muscular contraction has been performed on vertebrate muscles. In general the results were found to apply to the muscles of scallops (*Pecten*, *Astropecten*, *Chlamys*), sea mussels (*Mytilus*), edible oysters (*Ostrea*, *Crassostrea*), and *Anodonta*.

A complex chain of events is involved in muscular contractions. I will consider only the high points. Glycogen appears to be the principal, if not the only source of energy in this process. Its content in the adductor muscles of bivalves varies from less than 1 to about 3 percent. The immediate source of energy for muscular contraction is not derived, however, from the breakdown of glycogen. Considerable quantities of phosphate are released by the organic compounds called phosphagens. These substances contain (Weber, 1958, p. 5) an energy-rich phosphate bond and, therefore, are the "stores of immediately available energy." Creatine phosphate, identified as a phosphagen of vertebrate muscle, does not occur in mollusks; its place is taken by arginine phosphate. Phosphagen decreases during contraction and is formed again during rest. After prolonged contractions the tissues of the fatigued muscle become acidic due to the accumulation of lactic acid. Glaister and Kerly (1936) found that iodoacetate, which inhibits the formation of lactic acid in *Mytilus* muscle does not materially interfere with muscular contraction.

The key substance involved in the energy transformation in the muscles is, however, adenosinetriphosphate (ATP); the presence of ATP is a prerequisite to contraction. According to Szent-Györgyi's theory ATP has a great affinity to myosin and is strongly linked to it. Excitation of the muscle implies the formation of actomyosin (from actin + myosin), a process which does not take place in the absence of ATP (Szent-Györgyi, 1951). Dephosphorylization of ATP to adenosinediphosphate (ADP) is believed to be the most important reaction closely connected to the liberation of energy in the contracting muscle. The function of ATP, according to Weber (1958) is twofold: "it acts as a contracting substance if it is split and as a relaxing and plasticizing substance if it is present without being split." The ATP used in contraction is restored "almost as rapidly as it is broken down by transphosphorylation of phosphagens."

Since the phosphorylization of ATP is the main stage in the energy-providing reaction in the muscle, it is of interest to know the splitting capacity of this compound in the adductor muscles. Investigation of this problem by Lajtha (1948) showed that the phosphatase activity is much lower in bivalve muscles (*Mytilus* and *Pinna*) than in rabbit muscle. Lajtha suggests that this is correlated with the slow working of the adductor muscle, which does not require the quick energy changes needed in the more rapidly functioning muscles of vertebrates and insects.

Chemical changes in the adductor muscle of the oyster (*C. commercialis*) were studied by Humphrey (1944, 1946, 1949, 1950), who demonstrated the presence of arginine phosphate and of several phosphorylated breakdown compounds of glycogen. The glycogen can be synthesized in both parts of the muscle from glucose-1-phosphate, but synthesis is more readily effected in the translucent portion.

In the glycolysis of the oyster muscle the glycogen breaks down in the presence of added potassium, magnesium, and DPN (diphosphopyridinenucleotide) and yields a mixture of pyruvic and lactic acids (Humphrey, 1949). The glycolytic ability of the adductor muscle of the oyster is several hundred times less powerful than that of rabbit muscle.

Studies of the glycolysis in extracts of the adductor muscle of *C. commercialis* (Humphrey, 1944) disclosed three essential facts: (1) phosphate, potassium, magnesium or manganese, and DPN are the essential parts of the system resulting in the production of acid; (2) lactic and pyruvic acids are produced simultaneously; and (3) acid production is inhibited by fluoride and iodoacetate. The glycolysis in oysters and other invertebrates still is not well understood, particularly with respect to the metabolism of pyruvate by oyster muscles.

The ATP present in the adductor muscle has a definite relationship to glycolysis. The amount of ATP in the muscle decreases when oysters are left out of water. From this observation Humphrey advances the hypothesis that the breakdown of glycogen provides the energy for the muscle to resist the pull of the ligament. He thinks that the regeneration of ATP proceeds through glycolysis, which continues under both aerobic and anaerobic conditions. Both conclusions require further corroboration.

NORMAL SHELL MOVEMENTS

Studies of shell movements can give valuable information regarding the physiological state of the oyster and its reactions to the changes of environment. The only type of motion that can be performed by an adult oyster consists of two distinct components: the contractions of the adductor muscle that bring the opposing valves together and may completely seal off the soft parts of the oyster, and the springlike action of the ligament that pushes the valves apart during the periods of relaxation. The purely mechanical action of the ligament is counteracted by the tonus of the muscle, which retains a certain degree of elasticity even in the state of maximum stretching. If the muscle is cut off at the maximum gaping, the valves are pushed farther apart by the elastic force of the ligament.

METHOD OF RECORDING

Oysters selected for long-term observation (several weeks or months) should be free of boring algae and animals. The surface of the shell is scrubbed with a metal brush, washed, and dried. The left valve is embedded in a rapidly setting mixture of cement, sand, and unslacked lime in proportion 1:2:1. Care should be exercised to keep the edges of the valves free of cement mixture and to wipe out and wash with sea water all excess material. Mounted oysters are left in the air at room temperature for 12 to 24 hours.

A small metal loop cut from a paper clip may be used to attach strings which lead to a recording lever. The two arms of the U-clip are bent horizontally, and the loop is placed on the clean, dry surface of the right valve and sealed in a vertical position by a few drops of hot colophonium cement. For recording the up and down movements of a valve heart and muscle levers available at scientific supply houses can be used. Adequate levers can be made of strips of appropriate length cut from a sheet of plastic and mounted on pivots of a small glass rod inserted in a hole drilled in the supporting arm. It is convenient to have at hand levers of various lengths so that records of shell movements of several oysters can be made simultaneously on one kymograph drum. Unless there are some special reasons for not changing the sea water during the observations, the oysters are placed in running sea water, and the temperature of the water is recorded on a thermograph and its salinity checked at regular intervals.

The records reproduced in this book were obtained by using a slow-motion kymograph. The uppermost position of the writing pen always corresponds in these tracings to the position of a completely closed right valve; the lower position of the line marks the various degrees of opening of the shell. The magnitude of the up and down excursions of the writing pen depends on the ratio between the two arms of the lever, the distance between the hinge ligament and the place of the attachment of the string, and the height of the oyster. The magnification of shell movements recorded in the Bureau's shellfish laboratory at Woods Hole varied from three to seven times the actual excursions of the valves. A baseline representing the position of the writing pen when the shell is completely closed (not shown in the records reproduced here) may be obtained by rotating the drum rapidly before beginning observations.

Under ordinary circumstances the opening and closing movements of the shell are so small that the corresponding up and down tracings on kymograph paper are relatively short and are not distorted by the actual movement of the lever, which on wider tracings describes an arc on the side of the rotating cylinder. In case of wide gaping produced by experimental stretching of the muscle the distortion becomes serious since the writing point at the bottom moves ahead of the time marker and draws a gentle slope instead of a steep curve. To avoid possible misinterpretation the true position of the writing lever at the time of maximum stretching and its return to the top as the muscle contracts are shown on the records by dotted lines.

For long-term observations the speed of the kymograph drum is adjusted to slow movement of about 1 inch per hour. When studies are made of the reactions of oysters to various stimuli the speed of the rotation should be increased to about three-eighths of an inch (1 cm.) per minute. With the fast- and slow-motion kymograph used in the Bureau's shellfish laboratory, the latter speed corresponded to one complete revolution of the drum per hour. With this technique several thousands of records of shell movements of oysters were obtained under a great variety of conditions using both normal and diseased oysters. Specimens used in the tests were taken from New England waters, Chesapeake Bay, South Carolina, the west coast of Florida, Mississippi, Louisiana, and Texas. A relatively small number of records were made of shell movements of *C. gigas* and *O. lurida* of the Pacific Coast. Many records were obtained while oysters were subjected to various types of poisons (chlorine, phenol, black liquor and red liquor of pulp mill wastes, crude oil, thiocyanates, etc.) or while they were given various concentrations of carbohydrates and suspensions of pure culture of *Escherichia coli*.

For a study of shell movements under normal conditions the oysters were kept in running sea water delivered at 10 times, at least, faster than the rate at which it was transported through the gills. Under this condition one can be certain that the products of metabolism were removed and the oysters were not deprived of food.

Shell movements play an essential part in the respiration, feeding, and rejection of silt, mucus, and excreta that otherwise may accumulate in the pallial cavity of the oyster. Material settled on the gills and mantle is rejected by rapid and powerful snapping of the valves. In addition to this rejection reaction there are smaller and slower changes in the tonus level of the adductor which may be interpreted as adjustments to a steady flow of water through the gills. It is not surprising that shell movements of oysters show great variations both in the rate and type of contraction. Analysis of the records made under known conditions in the laboratory indicates that in spite of this variability the movements of individual oysters can be grouped into five major types characterized by their responses to various conditions.

FIVE MAJOR TYPES OF SHELL MOVEMENTS

In comparing the records of shell movements it is necessary to know the following essential points: the highest and lowest level reached by the writing pen during the periods of closing and opening of the valves, the frequency at which the contractions occur, and the speed of rotation of the drum. Published reports frequently fail to mention these significant details. Another feature of importance is the general level corresponding to the tonus of the muscle to which the valve returns after each brief closing. Under normal conditions the adductor muscle is never completely relaxed. The distance to which the valves are pushed apart by the hinge ligament is, therefore, indicative of the degree of relaxation.

During my years of study, more than 2,000 tracings of shell movements of oysters were obtained under a great variety of conditions. It was possible to group them into five principal types which for the sake of brevity are designated by the first five letters of the alphabet.

Type A

The three curves shown in figure 153 (A–1, A–2, and A–3) indicate normal behavior of the oyster. The differences in the appearance of the curves are due primarily to differing speeds of drum rotation. Curve A–2 is a continuation of curves A–1 with the drum movement reduced from 15.3 cm. to 3.6 cm. per hour. The extreme right portion of curve A–2 indicates the summation of several stimuli that caused brief closing of the valves. The curve A–3 is a variation of type A–1 and is essentially similar to curves A–1 and A–2. The writing lever in curve A–3 was set in such a way that the magnification of the vertical excursions was only one-third of that used in curves A–1 and A–2. The contractions were, however, more frequent. Several downward excursions of the pen indicate brief attempts to widen the opening of the valves, but the general tonus level of the adductor remained fairly constant.

Type A shell movement, shown in figure 153, represents movements of an undisturbed oyster that maintains a steady current of water for the ventilation of the gills and for the collection of food. The general level of opening of the valves is fairly constant (curves A–1 and A–2). Relaxation of the muscle immediately after rapid contraction is slow, and the resulting curve slopes down gently (see right parts of curves A–1 and A–2). Sudden snapping of the valves is associated with the discard of rejected food, mucus, detritus, and other particles that accumulate on the inner surface of the pallium. This rejection reaction is an important feature of oyster behavior for it is the principal method of keeping the pallial cavity free from the accumulation of foreign matter.

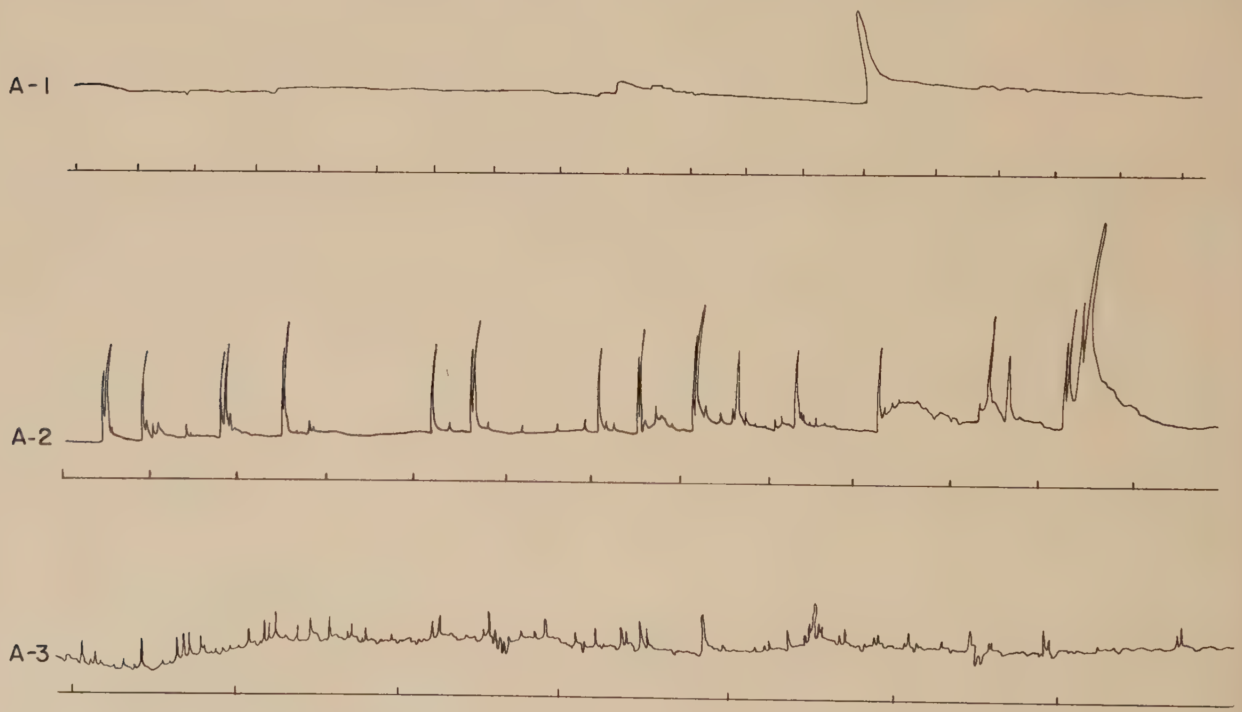

FIGURE 153.—Shell movements of normally feeding oyster. Type A. Vertical magnification of curve A–3 is about one-third of that in A–1 and A–2. In each curve the uppermost point corresponds to the position of the lever when the shells are completely closed. Time interval: A–1, 5 min.; A–2, 30 min.; A–3, 1 hour.

Numerous minor contractions (fig. 153, A–2) that occur between the rejection reactions only slightly reduce the opening between the valves and are more difficult to interpret because they are not accompanied by the discharge of any material. Possibly they represent the fine adjustments made by the oyster in maintaining a steady flow of water through the gills. On the other hand, it is also possible that they are responses to minor physical disturbances such as vibrations of laboratory floors and slight changes in illumination. None of the existing laboratories in the United States have the shockproof floors and walls that would assure complete elimination of the outside disturbances caused by street traffic and footsteps within the building.

Type B

Type B shell movement is characterized by the increased frequency and well-pronounced periodicity of contractions and corresponds to the state of increased excitability (fig. 154). Curve B–1 was observed in oysters which were exposed to a rapid rise of temperature from 13° to 25.6° C. B–2 represents the behavior of oysters affected by the metabolites accumulated in stagnant and unaerated water. The uniform and rapid contraction shown in B–2 stopped immediately when the water was changed.

Curve B–3 represents a similar activity recorded on a rapidly moving drum. The relaxation periods are much shorter, but the level of the muscle tonus remains constant. Shell movements of this type were frequently observed in oysters which were left after spawning in water containing large quantities of oyster eggs and sperm. Normal movements of the type A–1 were resumed as soon as the water was changed.

Type C

The curve of type C shell movements (fig. 155) illustrates periods preceding or following changes in the degree of opening and closing of the valves. Both periods are characterized by a series of minor contractions and relaxations until the final tonus level is reached. The type shown in C–2 (left part of the curve) is a typical "staircase" or "Treppe" reaction of the adductor muscle, which contracts in several distinct stages. This reaction is the response to an irritating substance added

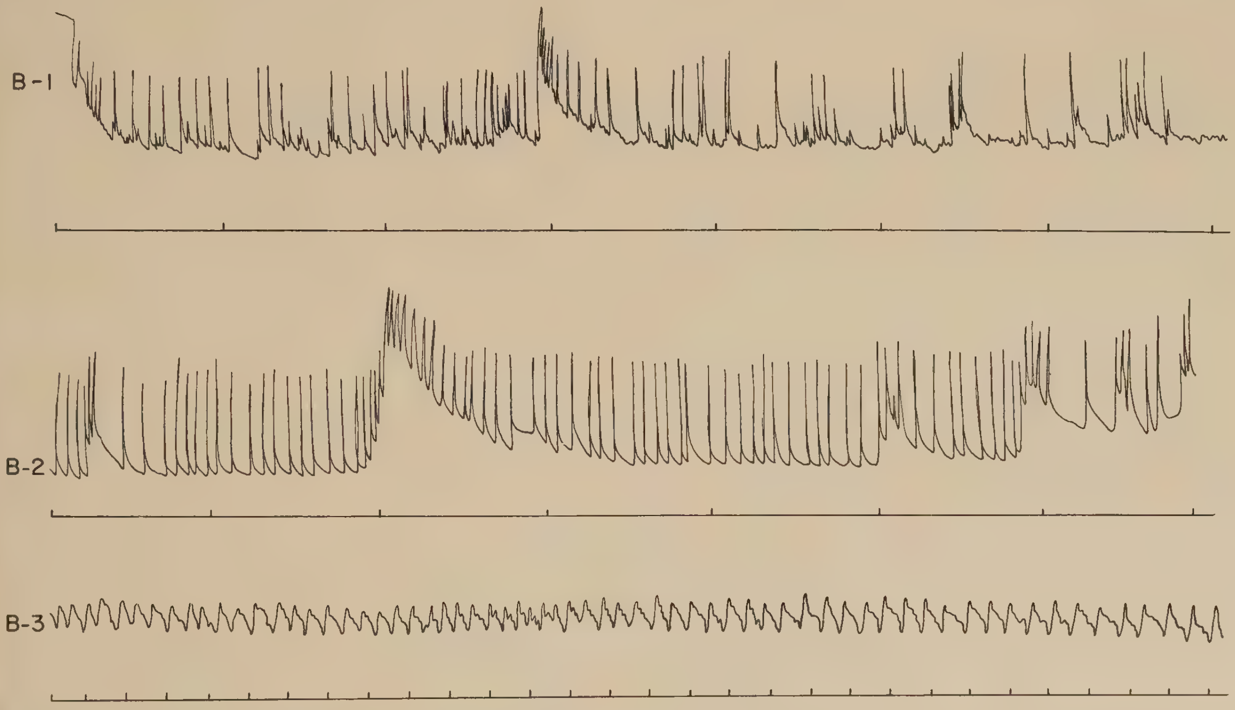

Figure 154.—Shell movements of type B are typical for the state of increased excitability frequently caused by the accumulation of metabolites in sea water or rapid rise of temperature. Vertical magnification in B–3 is about one-fourth of that in B–1 and B–2; uppermost points correspond to closed shells. B–1 temperature increased from 13° C. at the start to 25.6° C. at the end. B–2, B–3 increased muscular activity due to the accumulation of metabolites. Time interval: B–1, 1 hour; B–2, 1 hour; B–3, 1 minute.

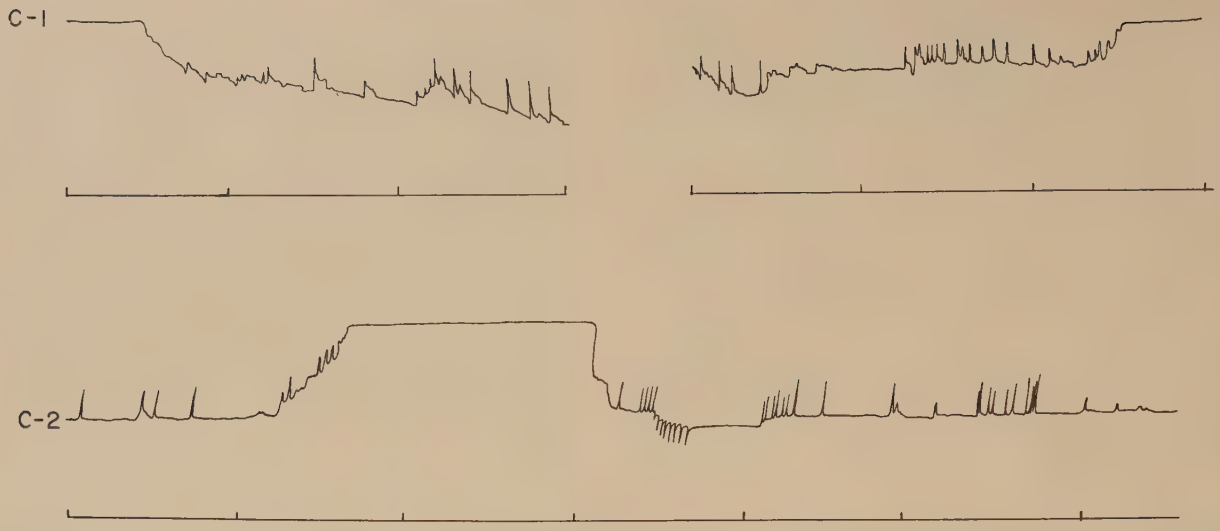

Figure 155.—Shell movements of type C, preceding the closing of valves (left side) and following their opening (right side). Note staircase movement in C–2. Time interval: C–1 and C–2, 1 hour each.

to the water. A "staircase" in reverse direction sometimes takes place during the opening of the valves (C–2, right half). This behavior was provoked by small doses of oyster sperm, vitamins, and sugars injected between the valves into the pallial cavity. The "reversed staircase" may be interpreted as a testing reaction of the oyster, which adjusts the opening of the valves to a needed rate of ventilation.

Type D

Shell movement of type D (fig. 156) was observed in oysters affected by various poisons which caused increased excitability of the adductor muscle (D–1). In case of a prolonged action of poison the periods of greater excitability (D–2 and D–3) are interrupted by gradually increasing durations of periodical closure (D–4 and D–5). This type of shell movement is a symptom of a highly advanced pathological condition resulting from poisoning, disease, or exposure to adverse physical conditions. It is typical for dying mollusks (D–5).

Type E

The E type of shell movement associated with the spawning of the female oyster is characterized by great regularity, rapidity, and rhythmic up and down strokes (fig. 157). At the beginning of the reaction the time needed to reach the maximum relaxation level is very brief, almost equal to the time of the contraction (see: E–2). During the relaxation phase (downward stroke) there is a brief period of slowing down in the decrease of muscular tension. On the curve this period is represented by a small plateau. This moment coincides with the passage of eggs through the gills into the pallial cavity. The eggs in the pallial cavity are dispersed into the surrounding water by rapid contractions of the adductor.

Shell movements that take place during the spawning of a female do not occur at any other time and cannot be induced by drugs. They cease with the cessation of spawning. The factor that induces female spawning (temperature or chemical stimulation by sperm) has no effect on the type of shell movement of a male and is ineffective on nonspawning females. It is probable that this type of muscular activity is associated with the discharge of eggs from the gonads.

DURATION OF PERIODS OF OPENING AND CLOSING

The length of time the shells remain open or closed and the conditions that affect this behavior are of importance to oyster biology. Obviously the normal functions of the organism, such as respiration, feeding, and elimination of waste products, can be performed only when the valves of the mollusk are open. It does not follow, however, that the opening of the shell indicates that the mollusk is feeding or is ventilating its gills. Under certain conditions water may be shut off from the pallial cavity by the pallial curtain or by the cessation of ciliary motion while the valves remain open. However, in the majority of laboratory observations of the behavior of oysters in unadulterated sea water the opening of the valves coin-

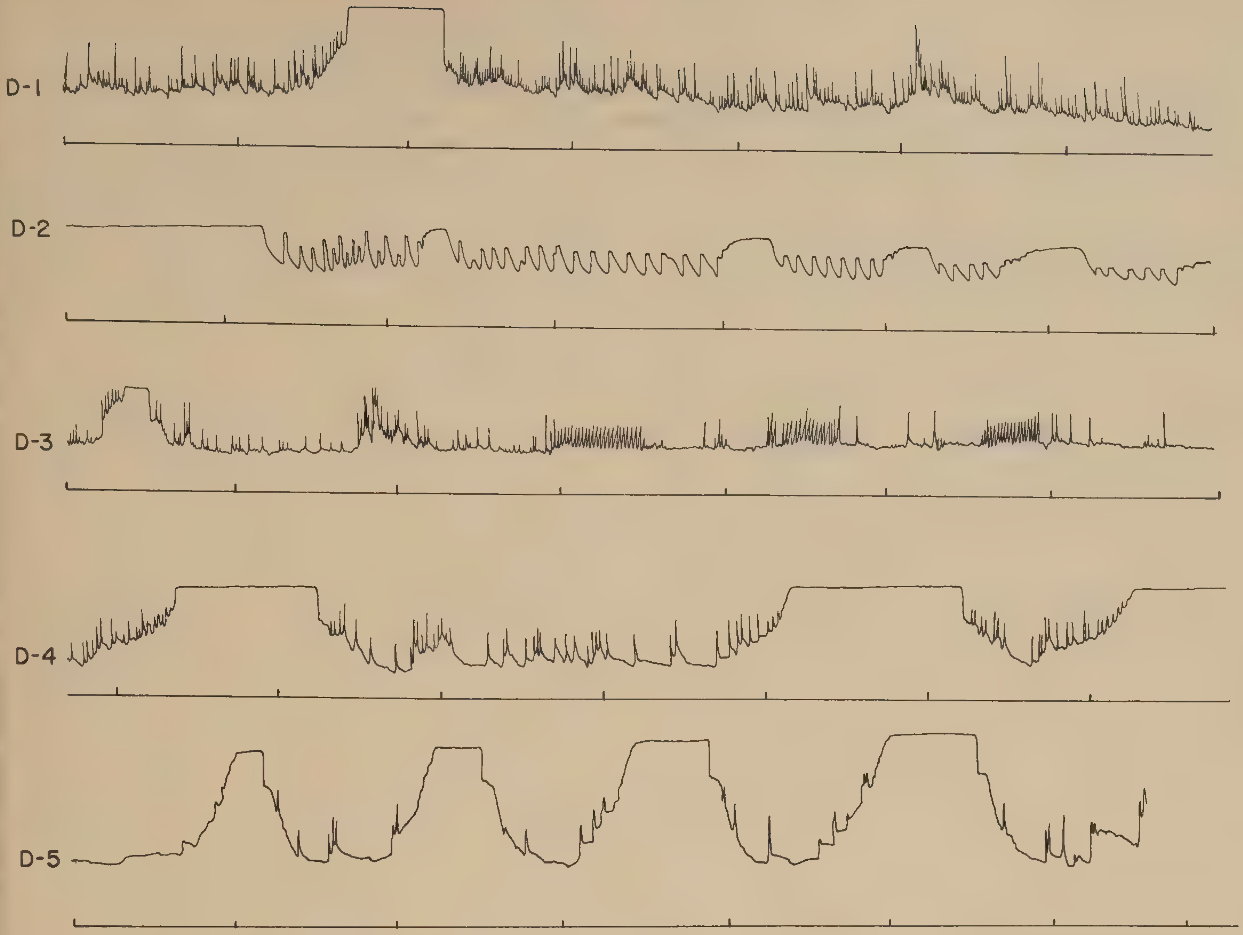

FIGURE 156.—Shell movements of type D are observed in the oysters poisoned by toxic substances or weakened by adverse environment. D–5 shell movements of a dying oyster. Vertical excursions of the writing pen are magnified three times in all tracings. Uppermost level of the curves corresponds to closed shells. Time interval: D–1, D–2, D–3, D–4, D–5, 1 hour each.

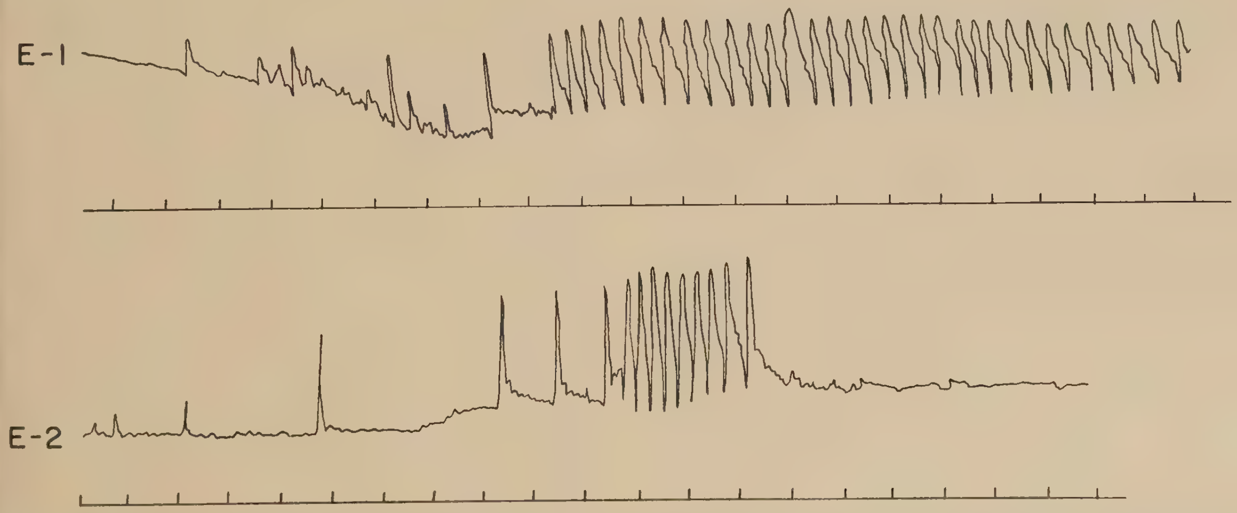

FIGURE 157.—Shell movements of a spawning female. Note the frequencies of up and down movements, brevity of the relaxation periods and slowing down at the middle of the downward strokes; this brief period coincides with the penetration of eggs through the gills. Time interval: E–1 and E–2, 1 minute each.

cided with the maintenance of a steady cloacal current.

The determination of the number of hours the oyster remains open under average normal conditions is of significance in studies of reactions of the mollusk to changes in its environment. Certain industrial wastes discharged in sufficient concentrations into natural waters reduce the time the oysters stay open. It was found, for instance, that the red liquor which is the waste product of pulp mills using acid digestion of wood and the black liquor of pulp mills which employ a sulfate process exert this effect on the Olympia oyster (Hopkins, 1931) and on *C. virginica* (Galtsoff, Chipman, Engle, and Calderwood, 1947). Any condition that forces oysters to remain closed for an abnormally long time deprives them from taking in food and eventually may harm them.

The percentage of time during each 24-hour period that the oysters are open can be used as an index of normal behavior, provided the shell movements of the mollusk do not indicate pathological conditions of the type shown in curves D–1 to D–5. Failure to recognize the significance of this type of shell movement while recording the time the oyster remains open may lead to serious misunderstandings and errors. Unfortunately there are many published data in which the "time open" was recorded without observing the character of shell movements.

The length of time *C. virginica* remains open is also influenced by temperature and by the state of the oyster itself. Since the shell movement is influenced by several external and internal factors, it is not surprising that there is a great discrepancy in the estimates of the average duration of "open shells" reported by various investigators.

In the Bureau's shellfish laboratory at Woods Hole from June 15 to October 15, 1926, 132 daily records of 34 oysters observed gave an average of 17 hours 7 minutes for open shells. The temperature of the water during this period ranged from 13° to 22° C., but daily fluctuations of temperature were insignificant, never exceeding 1.5° C. (Galtsoff, 1928). Records of the three oysters kept by Nelson under observation for 21 days in New Jersey water indicated that the shells remained open on the average of 20 hours per day at temperatures varying between 22° and 25° C. (Nelson, 1921). For oysters kept in running sea water at a Beaufort, N.C., laboratory, average time open in October to November varied between 10 and 14 hours (Hopkins, 1931). The temperature of water was not recorded. Two hundred and one daily records of 49 York River (Virginia) oysters kept under observation in the laboratory at Yorktown showed that the periods of opening varied from 19.2 to 24.0 hours a day (Galtsoff, Chipman, Engle, and Calderwood, 1947). Within the temperature range of 17.0° to 28.0° C. Long Island Sound oysters were found to remain open for an average period of 22.5 hours. The latter data are based on 64 records of 18 oysters (Loosanoff and Nomejko, 1946). *O. lurida* of the Pacific Coast remained open for an average of 20 hours a day at the temperature range of 5° to 17° C. (Hopkins, 1931).

A sample of oysters always includes several individuals that may remain closed for 24 hours or longer. One or two of them will reduce unduly the average figure based on a small number of observations. Furthermore, under identical conditions of the normal environment (i.e., not affected by pollution, dredging, or other disturbances) an oyster may keep its shell open or closed for varying periods of time depending on the requirements of the organism for food and oxygen. I found that immediately after spawning the female oysters have a tendency to keep their shells closed for several days. On the other hand, oysters left overnight out of water open almost immediately upon being returned to sea water. It is reasonable to assume that they accumulated an oxygen debt during the period of closure. In view of these observations the differences in the duration of periods of opening or closing described for oysters of different localities have no particular significance. The average value may be useful, however, in determining the adverse effects of the changes in the population of oysters in a given locality and in making a comparison between the behavior of these individuals in clean and polluted waters.

EFFECT OF TEMPERATURE

Temperature as such has no direct influence on the duration of shell opening. There was no significant difference in the length of time the Woods Hole oysters remained open when kept at temperatures varying from 15° to 30° C. (Galtsoff, 1928). It is rapid change in temperature, often occurring in those laboratories where sea water is subject to wide diurnal fluctuations, that has a pronounced effect on shell movements. *O. lurida*,

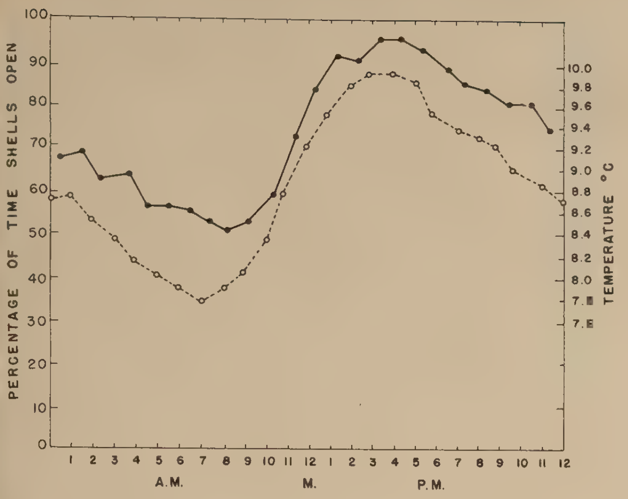

FIGURE 158.—The average percentage of time open of two specimens of *O. lurida* at each hour of the day observed over the 29-day period (solid circles). Average temperature readings for each hour during the same period (open circles). From Hopkins (1931), fig. 4, p. 6.

for instance, has a tendency to close with the falling of temperature and open with a rise of temperature (Hopkins, 1931). The sensitivity of this oyster to temperature changes was reported to be greater at the lower range. At 4° to 6° C. the oysters remained closed a relatively high percentage of the time; at 6° to 8° C. they were open only about 6 hours, while at the maximum of about 15° C. they remained open over 23 hours per day. In both cases the diurnal curve of shell activity was parallel to the curve of temperature fluctuation observed by Hopkins (1931) although the percentage of time open in warmer water was consistently higher (fig. 158). It would be of interest to repeat these observations and compare them with controls kept at a constant temperature since Hopkins' temporary laboratory near Olympia, Wash. lacked adequate equipment for regulation of temperature. He concluded that "change of temperature is more important in affecting the length of time Olympia oysters remain open than the degree of temperature itself." The results of his observations on *C. virginica* at Beaufort, N.C., bear close resemblance to those described above but, unfortunately, they were not accompanied by thermograph records and so are not entirely convincing. His conclusions need clarification.

EFFECT OF LIGHT AND DARKNESS

Periods of light and darkness have no apparent effect on the closing or opening of the valves. Analysis of 103 daily records of shell movements of oysters kept in the Bureau's Woods Hole laboratory in running sea water at nearly constant temperature (daily fluctuations ±0.5° C.) and constant salinity shows that of the total number of 831 hours of inactivity (shell closures), 266 hours or 32 percent occurred during the 8-hour period of darkness and the balance of 565 hours, or two-thirds of the total, took place during the remaining two-thirds of daylight (Galtsoff, 1928). During the summer, from June to August inclusive, the Long Island Sound oysters kept their shells open for 94.4 percent of the total time during daylight and 93.8 percent during the hours of darkness (Loosanoff and Nomejko, 1946). These observations repudiate Nelson's conclusion that the periods of inactivity (or closings) occur during darkness (Nelson, 1921, 1923c).

EFFECT OF TIDE

There is no evidence that the opening and closing of oyster valves is related to the stages of tide. The idea that oysters living below the low-water mark are relatively inactive during the outgoing tide and that the times of cessation and commencement of feeding are correlated to stages of the tide, was several times expressed by Nelson (1922, 1923a, 1923c, 1938) and without verification was accepted by Orton in his article in Encyclopedia Britannica (Orton, 1929). Loosanoff and Nomejko (1946) analyzed the kymograph tracings of shell movements of oysters kept under virtually natural conditions on a platform installed on a small oyster bed on the bottom of Milford Harbor in Long Island Sound. They found that the shells remained open on an average of 93.4 percent of the time during the flood periods and 95.2 percent of the time during the ebb periods. The tidal changes in Long Island Sound are not accompanied by the excessive changes in the temperature, salinity, pH, and turbidity of water which frequently take place in the tidal streams of the southern Atlantic states and may influence the shell movements of oysters.

POWER OF THE ADDUCTOR MUSCLE

Anyone who attempts to open a live oyster by inserting and twisting a knife between the two valves becomes aware of the considerable resistance exerted by the mollusk. As a rule the valves of healthy oysters just taken out of sea water are difficult to pry apart. The power of the adductor muscle, which is solely responsible for keeping the valves tightly closed, varies greatly in oysters of the same size and environment. Prolonged exposure to air so weakens the adductor that oysters left out of water for several days can be easily opened.

In attempts to measure the power of the adductor of various bivalves Plateau (1884), Marceau (1905a, 1905b), and Tamura (1929, 1931) drilled holes near the edge of the shells and inserted rods or hooks to which they attached weights. The opposite valve was immobilized. Assuming that the adductor muscle is an elastic body, the amount of work (W) done by the adductor against the loaded weight (G) was calculated by using a simple formula $W=\frac{ac}{ad}G$ where ac is a distance in centimeters from the ligament to the attachment of the weights; ad is the distance in centimeters from the ligament to the center of the adductor muscle; and G is the weight in grams applied to the valve. Under a known pulling force the shell movements were traced on a kymograph and a record was made of the time and load under which the muscle fibers were torn off. Continuous irritation of the adductor by the foreign body (hook or rod) inside the shell near the mantle makes this technique objectionable. Furthermore, the end point of the experiment, the tearing off of the muscle, is of no biological significance compared to a determination of the tensile force of the muscle fibers.

The method used in the Bureau's shellfish laboratory eliminates these objections. The left valve of the oyster is mounted on a heavy cement block, using a very strong mixture of portland cement and sand to which a small amount of plaster of paris is added (fig. 159). The base is bolted to the frame D which may be placed in the aquarium tank B supplied with running sea water. A galvanized iron screw (a) about 1 inch in length is inserted into the valve at the center of the attachment of the adductor muscle. Its tip should not penetrate the valve. Enough portland cement or other highly adhesive mixture is applied to the shell surface around the screw to make a cone of about 1 inch in diameter; the top of the screw (a) should protrude above the cement. A metal stirrup (E) consisting of a pair of iron bars (b) with pronged arms at the lower end and a hook (d) mounted at the upper end connect the valve and the pan (e) of the laboratory balance

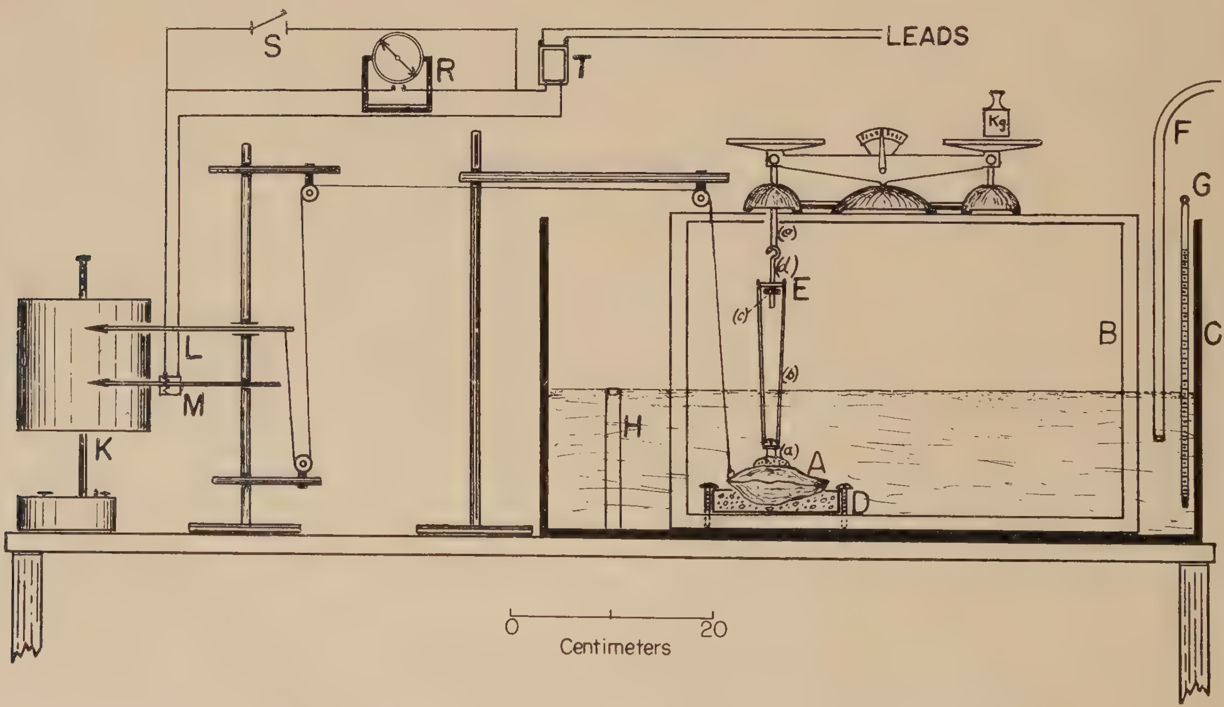

FIGURE 159.—Method of determining the resistance of the adductor muscle of *C. virginica* to a pulling force. A—cement base, bolted to wooden frame D and placed in tank B; a—galvanized iron screw; b—bars of the stirrup E; c—adjusting nut; d—hook for connecting the stirrup to the balance; e—left pan of the balance; F—seawater intake; H—overflow; K—kymograph; L—writing lever; M—signal magnet and pen; R—Telechron timer; T—transformer.

placed on frame D. The length of the hook is adjusted by turning the nut (c). The two pans of the balance are placed in a zero position, and the desired weight is put on the right pan. The right valve of the oyster is connected to the writing lever (L) of the kymograph (K). The writing pen (M) is attached to a signal magnet which is activated by an electric timer (R) and transformer (T). The timer is made by mounting a plastic disc on the axis of a Telechron motor making one revolution every hour. A short piece of copper wire at the periphery of the disc, indicated in figure 159 by the arrows, completes the circuit every 30 minutes (at the vertical position of the arrow). The weight of the balance is sufficient to keep the platform from floating when it is placed under water. Sea water is supplied through the intake (F); the overflow (H) controls the water level. This setup was successfully used in a large number of tests made both in the air and under water.

Occasionally the bond between the cement cup and the surface of the shell was insufficient for a pull of 8 to 10 kg. and had to be adjusted by using a stronger mixture and slightly roughening the surface of the valve. In the majority of cases the connection between the valve and the cement cap remained intact even when the pulling force of about 10 kg. was applied and occasionally the muscle itself was torn in the middle.

The purpose of the test was twofold: to study the behavior of the adductor under variable pulling force and to determine the time required to cause the loss of tonus by the muscles that were being stretched by weights varying from 2 to 10 kg. directly over the muscle scar.

New England oysters kept in the harbor near the laboratory were used in all the tests. The oysters were about 5 inches in height and appeared to be in good condition with the shells undamaged by boring sponge.

TESTS MADE IN AIR AND IN WATER

Adult oysters exposed to air at room temperature are able to withstand the pulling force of several kilograms for several days. Under the weight of 7 to 8 kg. the adductor muscle opened immediately (fig. 160). A force of 8 kg. (2,185.8 g./cm.^{2} of cross-sectional area of the adductor) caused immediate stretching of the adductor to about one-third of the maximum gaping distance, which was attained within 6.5 hours. During the 5 hours following the initial stretching there was no shell movement but the adductor retained its tonus level; the response to pricking (several small upward strokes on the record) was very slight. Final stretching to 1.5 cm. gaping distance of the valves was relatively rapid. At this stage the adductor lost the tonus and failed to respond to stimulation. Upon removal of the weights the muscle regained its elasticity and contracted (right side of fig. 160). For several hours an oyster weighing only 18.2 g., exclusive of shell, was capable of maintaining a constant tonus level against the pull of 8 kg.

In all tests in which the pulling force of 10 kg. per oyster was applied (from 2.5 to 3.0 kg./cm.^{2} of the muscle area) the muscle stretched immediately and the gape of the valves reached the maximum width of 15 to 18 mm. The muscle failed to respond to pricking or to the application of 0.1 *N* hydrocholoric acid but retained a certain degree of elasticity and was able to counteract the pulling force of the ligament. As soon as the muscle was cut off the valves opened several millimeters beyond their former position.

Individual variations in the time required for a muscle to reach maximum stretching are considerable. The time needed to produce tonus loss is inversely related to the weight applied to the valves. The pulling force of 0.5 kg. (131 to 136 g./cm.^{2}) applied for 15 days had no effect on the opening of the oyster shell in the air (at room temperature of 15° to 18° C.). At the pulling force of about 500 g./cm.^{2} the loss of tonus and failure to respond to stimulus developed in 300

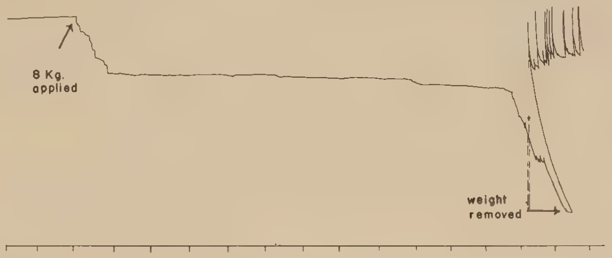

FIGURE 160.—Record of shell movement of *C. virginica* kept in air under the pulling force of 8 kg. (2185.8 g./cm.^{2} of cross-sectional area of the adductor muscle). Arrows indicate time when the weight was applied (upper left) and removed (lower right). Temperature 18° to 23° C. Total weight of oyster meat 18.2 g.; of shell 166 g. Maximum gap (right end of the curve) 1.5 cm. The distortion of the lowermost position of the lever with reference to the horizontal axis is marked by the heavy arrow. Time interval: 0.5 hour.

hours. To avoid desiccation the oyster in this experiment was surrounded by a small moist chamber. With the increase in weight the time of complete loss of tonus rapidly decreases (fig. 161).

Muscles which were kept for several hours under a pulling force of about 1.7 kg./cm.² of cross-sectional area suffered a temporary injury which resulted in abnormal shell movements after the return of the oysters to sea water (fig. 162). The two tracings reproduced in this figure are almost identical, although in the case of oyster A a pulling force of 6 kg. was used while 8 kg. were applied to oyster B. In both instances the pulling force per unit of muscle scar area was the same, 1,676 in A and 1,675 g./cm.² in B. After a few days in running sea water both oysters completely recovered and their shell movements became normal.

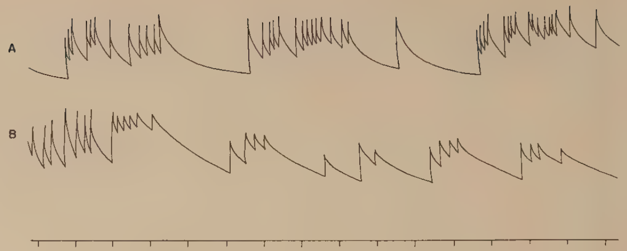

Figure 162.—Shell movements of *C. virginica* in sea water after the removal of weight of 6 kg. or 1,676 g./cm.² of muscle area (upper line) and 8 kg. or 1,675 g./cm.² of muscle area (lower line) applied to the valves. Weights were removed after complete loss of tonus was attained in 43.52 and 52 hours exposure in air at temperature 23° to 24° C. Water temperature 13.5° C. Time interval: A and B, 0.5 hour each.

In oysters kept in sea water the relationship between the weight applied to the valves and the time needed to attain tonus loss is less regular and individual differences are much greater than for oysters left in the air. With a pulling force of about 1.5 kg./cm.² of muscle area some of the oysters showed tonus loss in less than half an hour while others remained closed for many hours. The relationship between the increasing pulling force and the time required to develop loss of tonus is shown in figure 163.

Changes in the character of shell movements of

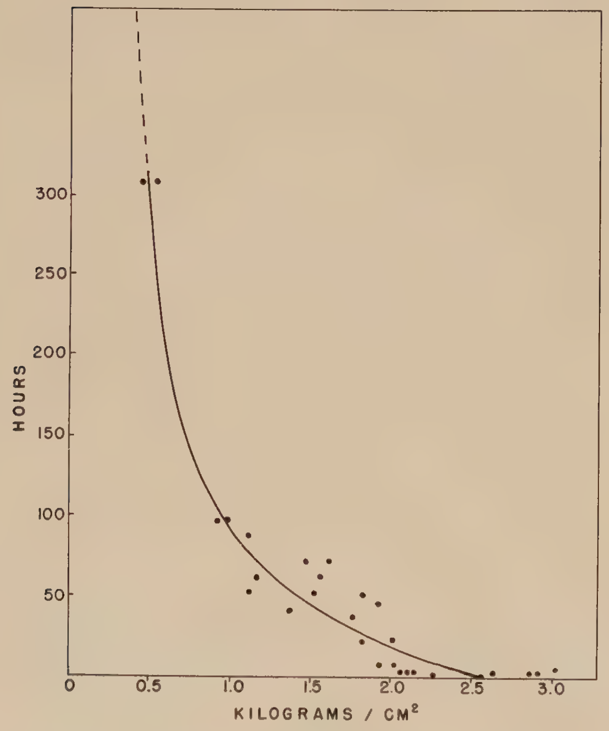

Figure 161.—Time in hours required to obtain loss of tonus of the intact adductor muscle kept under constant pull in kg./cm.² of the cross-sectional area of the adductor. Experiments with *C. virginica* kept in air at temperatures between 18° and 24° C.

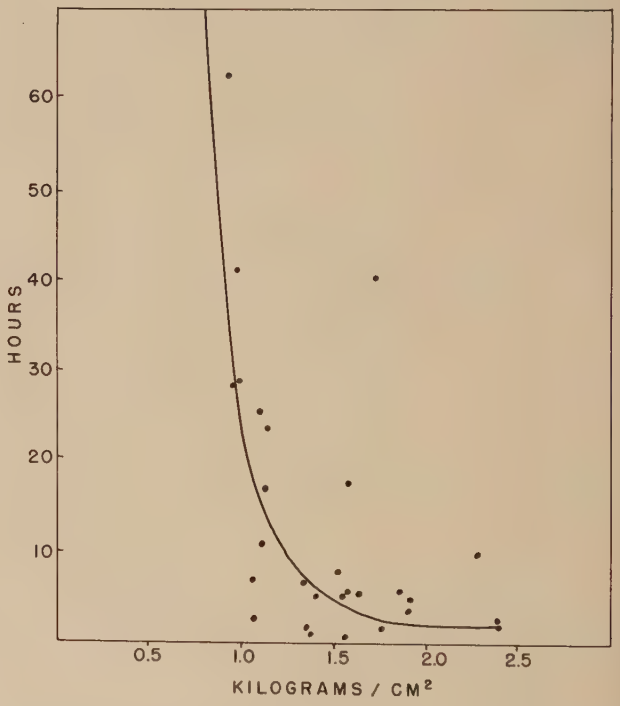

Figure 163.—Time in hours required to obtain complete loss of tonus in the adductor muscle of *C. virginica* kept in water under a constant pulling force expressed in kg./cm.² of the cross-sectional area of the adductor muscle. Temperature 13.9° to 18.0° C. At the pulling force of 0.59 kg. complete tonus loss was obtained in 274 hours (11.5 days).

an oyster kept under the continuous pull of the relatively light weight of 2 kg. (606 g./cm.^{2} of cross section of muscle area) are shown in figure 164. The five lines represent excerpts of about 7.5 hours duration from a continuous recording made at a temperature of 13.9° to 14.1° C. and salinity of 31.3 $^{\circ}/_{\circ\circ}$. In line A the movements are normal. Their amplitude is increased after the application of a pulling force of 2 kg.; at the same time the frequency of contraction decreases (line B). This condition continues until the 67th hour (line D, middle part) when the muscle begins to stretch and the number of contractions greatly increases. At the 71st hour (end of line D) the muscle does not respond to stimulation. After removal of the weight (line E) shell movements are restored. The frequency of contractions during the recovery period is greater than under normal conditions. Within the next 48 hours normal shell movements of the type shown in line A are resumed.

Similar experiments in the air at higher temperatures varying from 18.5° to 24.0° C. gave slightly different results shown in figure 165. The pulling force of 2 kg. per oyster applied in this case was equivalent to 590 g./cm.^{2} of the cross-sectional area of the muscle. Loss of tonus was attained in this case after 274 hours (line C) when the gap between the valves reached the maximum of 1.5 cm. Pathological condition of the muscle was apparent after 96 hours (line A) and became pronounced at 190 hours (line B). After removal of the weights the oyster was left in running sea water but failed to recover and died in 2 days.

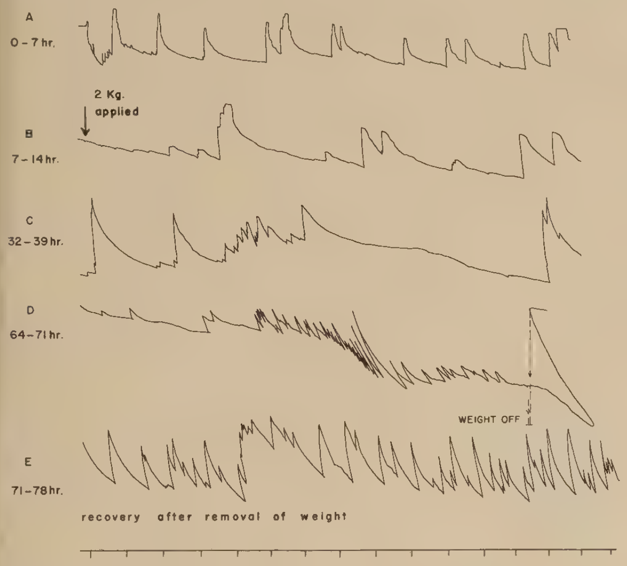

FIGURE 164.—Shell movements of *C. virginica* in sea water under continuous pull of 2 kg. (606 g./cm.^{2} of cross-sectional area of the adductor muscle). Temperature 13.9° to 14.1° C. Salinity 31.3 $^{\circ}/_{\circ\circ}$. A—normal shell movements before the application of weight. B—immediately after the application of weight. C—after 32 hours; note increased gaping. D—after 64 hours; wide gaping, complete loss of tonus and lack of response to stimulation. Maximum valve opening 1.5 cm. E—increased muscular activity during the recovery period following the removal of the weight. Time interval: A, B, C, D, and E, 0.5 hour each.

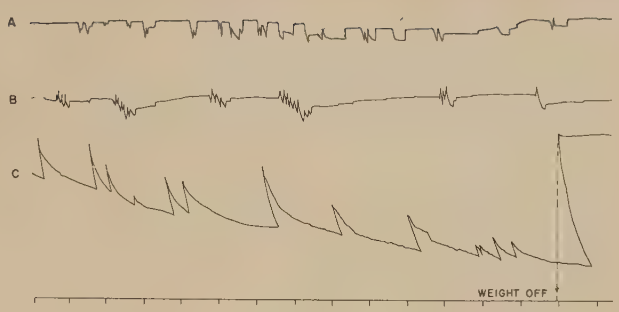

FIGURE 165.—Excerpts of the continuous records of shell movements of *C. virginica* in air under the pulling force of 2 kg. (590 of the cross-sectional area of the adductor). A—line ends at the 96th hour after the application of force; B—at 190th hour; C—at 274th hour when the muscle failed to respond to stimulation. Widest gap of valves 1.5 cm. Temperature 18.5° to 24° C. Time interval: A, B, C, 0.4 hour each.

A lighter weight (315.5 g./cm.^{2} of muscle area) applied to an adult oyster kept in running sea water at temperatures ranging from 13.9° to 18.0° C. produced very slow changes in the normal shell movements (fig. 166). The upper line of figure 166 represents normal movements recorded immediately after the application of the weight. A noticeable increase in the amplitude of contractions began on the 3rd day and continued through the 11th and 12th days. During the 13th and 14th days the amplitude of up and down strokes was greatly reduced; loss of tonus and failure to respond to stimulation developed by the 18th day. The last line shows the typical staircase contraction following the removal of the weight, indicating that the muscle retained some of its ělasticity. At the maximum amplitude of the contractions (9th and 11th days) the oyster periodically lifted the weight of 1 kg. to the height of about 1 cm. Ten days after the end of the test the oyster recovered completely and its shell movements became normal.

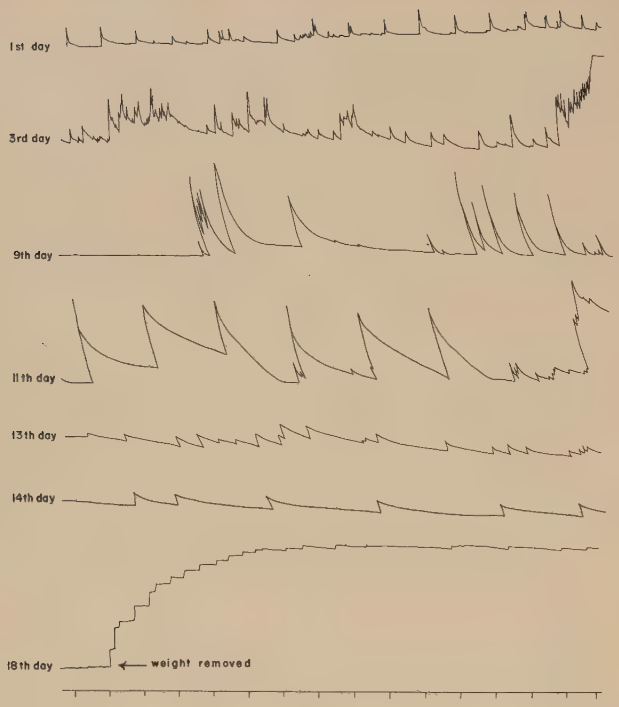

Figure 166.—Shell movements of *C. virginica* in running sea water under a continuous pull of 1 kg. (312.5 g./cm.^{2} of the cross-sectional area of the adductor muscle). Temperature 13.9° to 18.0° C. Time interval: 0.5 hour.

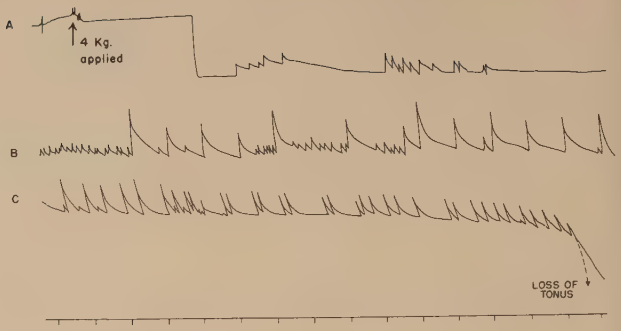

Figure 167.—Shell movements of two Cotuit oysters, *C. virginica* (lines A and B), and *C. gigas* (line C) in sea water under a continuous pull of 4 kg. or about 1,150 g./cm.^{2} of cross-sectional area of the adductor muscle. Temperature 14.5° to 16.5° C. Salinity 32.0 to 32.3 °/oo. The exact time of tonus loss is shown by the broken line and arrow. Time interval: A, B, and C, 0.5 hour each.

If much greater weight (4 kg. per oyster or 1,150 g./cm.^{2} of muscle area) is applied shell movements become abnormal at the very beginning of the test. This is demonstrated in the records of two Cotuit (Mass.) oysters (*C. virginica*) and one *C. gigas* shown in figure 167.

The stretching of the adductor muscle by a pulling force not exceeding 4 kg. per oyster did not interfere with their feeding; a strong current was maintained by the gills, and the feces were formed and discharged in a normal way. However, the secretion of mucus by the mantle and gills was greatly increased. Vast quantities of slimy material accumulated at the mantle edge and were discarded as pseudofeces.

The resistance of the adductor muscle to a pulling force exceeds by many times the force required to overcome the elasticity of the ligament and close the shell. This additional force is apparently needed to keep the valves hermetically sealed. The ability to keep the valves tightly closed has definite survival value. Mollusks possessing it are able to protect themselves against desiccation when exposed to air, or against adverse conditions caused by the presence of toxic substances in the water. Powerful muscular mechanism also helps them to resist attacks of starfishes, crabs, and other enemies that attempt to pry open their valves.

CYCLES OF SHELL MOVEMENTS

There is no indication of any periodicity in muscular activity in the kymograph records of shell movements of oysters that were kept in running sea water in the laboratory or kept outside on a suitable platform submerged from a pier (Loosanoff and Nomejko, 1946). Brown and his associates (Brown, 1954; Brown, Bennett, Webb, and Ralph, 1956) claim, however, that *C. virginica* possesses a persistent lunar cycle of activity with the maxima occuring at about 12.5 hour intervals. Oysters used for obtaining tracings of shell movements were kept for a fortnight or longer in about 4 or 5 l. of sea water which was not changed but was adjusted by occasional addition of distilled water to compensate for evaporation. The mean daily cycles were calculated for 15-day periods by obtaining the average value of opening for each hour of the day and applying to the data a very complicated method of adjustment. The main conclusions reached by the authors were that: (1) oysters and quahogs display "statistical rhythms of opening of shell while the overt rhythms are not apparent from kymograph records", (2) short periods of opening tend to occur about 6:00 a.m. and more or less prolonged periods of openings happen through much of the remainder of the day. The observations and their mathematical treatment are of interest from a theoretical point of view, but the ecological significance of the times of maxima and minima of activities in the daily cycle of the oyster are difficult to imagine at the present time.

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CHAPTER IX

TRANSPORT OF WATER BY THE GILLS AND RESPIRATION

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All bivalves maintain a steady flow of water through their gills for feeding, respiration, and the removal of products of metabolism. In the literature on oyster physiology this process is described under such names as pumping, filtration, ventilation, respiratory current, and feeding. When "feeding" is used in reference to the transport of water by the gills, the term is misleading since feeding implies the acceptance of food by the organism and involves sorting and rejection of particles removed from the water by filtration. The terms ventilation and transport of water appear to be suitable expressions for denoting the processes by which the oyster maintains a flow of water in a complex system of water tubes and through the epibranchial chambers. Both terms are used in this text interchangeably, depending on whether the emphasis is on collection of food or on respiration.

Maintenance of a steady stream of water and respiration are the two principal functions of the gill. Of lesser importance are the excretion (through diapedesis) of certain products of metabolism, the absorption of substances dissolved in water, and the ingestion of particles settled on the gills by the leucocytes present on their surface.

Water transport and oxygen uptake are interdependent functions that may be considered as two phases of a single process. It is convenient, however, to discuss them separately, keeping in mind that both activities occur simultaneously.

TRANSPORT OF WATER

The preceding chapter described how a steady rate of water current inside the demibranches is produced by synchronized beats along the thousands of tracts of lateral cilia. Temporary cessation of ciliary motion along some of the tracts or a disturbance in their rhythm of beating will result in a drop of hydrostatic pressure inside the water tubes and cause a leak of water through the ostia. This disturbance may slow down or stop the cloacal current.

The adductor muscle, the edge of the mantle, the gill muscles, and the ostia all play a part in the regulation of the flow of water produced by ciliary activity. It is self-evident that, within the limits determined by the capacity of the gills' chambers, the volume of water transported depends on shell movements and the width at which the two valves are kept apart. When the shell closes the current stops. A similar effect may be produced by the edges of the mantle independently of the movements of the valves. Sometimes the pallium assumes a vertical position and the tentacles along the edges of the opposing mantles interlock while the valves remain wide open. Under this condition no water penetrates through the curtain which seals the entire pallial cavity. This behavior occurs in the presence of low concentrations of toxic substances and during the spawning of the female oyster (ch. XIV, p. 304). It is, therefore, obvious that shell movements should not be considered as indications of the feeding of the oyster, a mistake which frequently is found in papers describing the "feeding" of oysters.

The rate of water transport may also be reduced by contractions of gill muscles bringing together the filaments of the plicae, followed by constriction of the ostia. The contraction of the ostia sometimes takes place independently of the contraction of gill muscles.

DETERMINATION OF THE RATE OF WATER TRANSPORT

The rate at which water is transported through the gills can be studied by both direct and indirect methods. In direct methods the volume of water discharged through the gills in a given time is collected and measured, or is calculated from observations of the velocity of the cloacal current. The indirect methods are based on determinations of the rate of removal of particles suspended in water. The powders used for the latter purpose include kaolin, natural silt, calcium carbonate, colloidal carbon, isolated chloroplasts, various plankton microorganisms such as *Euglena*, *Chlorella*, and *Nitzschia*, and cultures of unicellular algae that have been made radioactive.

Direct methods

There are two groups of direct methods. First, the water discharged by the gills may be intercepted, its volume measured, and the sample retained for analysis. In the second method the velocity of the current is determined by measuring the angle of deflection of a light paper cone or glass plate placed in front of the current. The rate of transport of water masses may be computed from the cross-sectional area of the column of moving water and the angular deflection of the plate. Both methods have certain advantages and disadvantages.

The methods which make it possible to collect and analyze water after it has passed through the gills are particularly suitable for studies of feeding and metabolism. The slight disadvantage is that the mollusk must be partially enclosed in rubber or plastic. In the second group of methods the mollusk is not in direct contact with any foreign material and is kept under normal conditions. The drawback is, however, that the discharged water can only be collected in very small quantities directly from the stem of the current inside the cloaca. Choice of method must be governed, of course, by the purpose of the observations.

Constant level tanks.—A very simple device consisting of two connecting vessels in which the level of sea water is kept constant was designed by Galtsoff (1926, 1928) and has been adapted and modified by many investigators making long-term observations on the feeding of the oyster. The apparatus, shown in figure 168, consists of two rectangular vessels, one large and one small, connected by a semicircular trough or by glass tubing of wide diameter inserted in the partition between the two vessels. The large vessel should be just big enough to accommodate an oyster mounted on a stand, the inlet tube for sea water, and a thermometer. The volume of the tank is made as small as possible in order to permit rapid exchange of water. Convenient dimensions for observations on adult *Crassostrea virginica* and *C. gigas*, are 10 by 6 by 4 inches for the large vessel, and 2 inches by 1½ by 2 inches for the small one.

The tanks are made of transparent plastocel, lucite, or similar nontoxic material about ¼-inch thick. The edges are sandpapered and cemented together by a solution of plastic in acetone, cellosolve, or by undiluted methylene chloride, and the small vessel is cemented to the side of the large one. After the cement is dry the tank should be washed carefully and rinsed in sea water. A semicircular trough made of thin plastic bent in hot water to the proper shape is mounted on the wall between the two vessels. The drain pipe is glass tubing, 6 to 8 mm. in diameter, inserted through the bottom of the small vessel; its upper end is slightly widened and adjusted to the exact level of water in the large vessel, which is controlled by a wide cut in the opposite wall of the vessel (left side in figure 168). The level of water is adjusted by moving the vertical overflow tube up and down (right side of fig. 168) until excess water running through the large vessel spills through the overflow and nothing enters the small vessel. Levels must be set very accu-

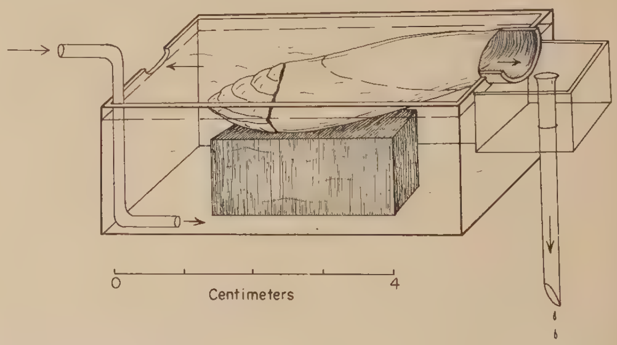

Figure 168.—Constant level tanks designed by Galtsoff to measure the volume of water transported by the gills of the oyster. Dimensions of the large vessel are 10 by 6 by 4 inches.

rately. When the correct height of the drain pipe is reached a few drops of water added to the contents of the small vessel will be discharged immediately. Setting the level of water in the small vessel too low may lead to serious error as the water will be forced through the gills by gravity. Since readings may be grossly influenced by poorly adjusted levels, repeated checking of the overflow must be performed at regular and frequent intervals.

The rate of supply of sea water into the large vessel should exceed the expected maximum rate of water transport by the oyster, which rarely exceeds 30 l. per hour. After equilibrium in the water levels in the two communicating vessels has been established, the tank is ready for use. Before the oyster is placed in the tank the mollusk is wrapped in thin rubber dam or in plastic sheeting cut in the shape of an apron. This technique, suggested first by Moore (1910), was adapted for oyster research by Nelson (1936, 1938). A triangular piece of sheeting cut to fit the shape and size of the oyster is spread on a table. After the valves are thoroughly scrubbed, washed, and dried, the oyster is placed on its left valve on the sheeting. The anterior half of the oyster is left free; only the posterior half is wrapped in the apron (fig. 168). The sheeting is attached to the shell with melted colophonium cement, starting with the lower (left) valve. Enough slack should be left at the edges of the shell and at the hinge side to allow free movement of the valve; small cotton balls are inserted in both places to prevent leakage of water. The two sides of the apron are then brought together to make a sleeve of the required diameter to fit the interconnecting trough of the tank and are joined using hot colophonium cement. A wide test tube is inserted in the apron and the apron sleeve is sealed by pressing the two sides of the apron against the glass while applying the cement. Before the oyster is put in the tank the apron should be tested against leakage by gently blowing air into the sleeve.

A small metal loop cut from the end of a paper clip and cemented to the flat valve is used to attach a string leading to the kymograph lever, which records the shell movements. The water levels in the tanks are checked again; the oyster is placed on a stand inside the tank and if necessary immobilized by plastic clay; the sleeve of the apron is drawn over the trough (or tubing) and is secured by a piece of string; the writing lever of the kymograph is connected to the valve. The last check for a possible leakage of water is made after the oyster opens its valves and begins to transport water. The escape of water through the seams of the apron can be detected by adding carmine suspension and watching the currents. In a correctly adjusted set all water transported by the gills enters the small vessel through the sleeve and can be collected, measured, and analyzed.

The transport of water can be recorded continuously by using various automatic devices, such as electric drop counters and dumping vessels. The latter turn over after the water has reached a certain predetermined level. Any of these devices can be connected to the kymograph lever which makes a mark on a rotating drum. The shell movements and the temperature of water are recorded simultaneously on the same sheet. The arrangement of various parts of an apparatus for recording the oyster activity and for collecting samples of water before and after its passage through the gills is shown diagrammatically in figure 169. The oyster in this diagram has been placed in a constant level tank A, which is provided with two connecting vessels B and C. A valve between A and B may be lowered to disconnect tank A, and cap L is used to close the connection between vessels B and C. Vessel B is slightly larger than C and is used for taking samples of water for plankton study or for gas analysis. The water in this vessel is protected from direct contact with air by the paraffined float H. The sample is taken through the drain tube X. The small vessel C has an overflow Y, which controls the levels of water in all three vessels. From the overflow Y water is delivered through tube Z to a dumping vessel E, which is mounted on a horizontal axis. A diagonal wall divides the vessel into two parts, of which only one (facing the reader) is being filled with water. Float F activates the system of levers and releases the catch L which holds the vessel in an upright position. The vessel overturns but snaps back into the position shown in the diagram. Proper adjustment of the vessel is obtained by attaching small weights (not shown in the diagram) to its bottom. The vessel with its levers is mounted on a solid frame D set on a heavy concrete platform. The water discharged at each dump activates the springboard G, which is connected to a writing lever N. Shell movements are recorded by the lever M; the electric time signal S connected to a timer T records time

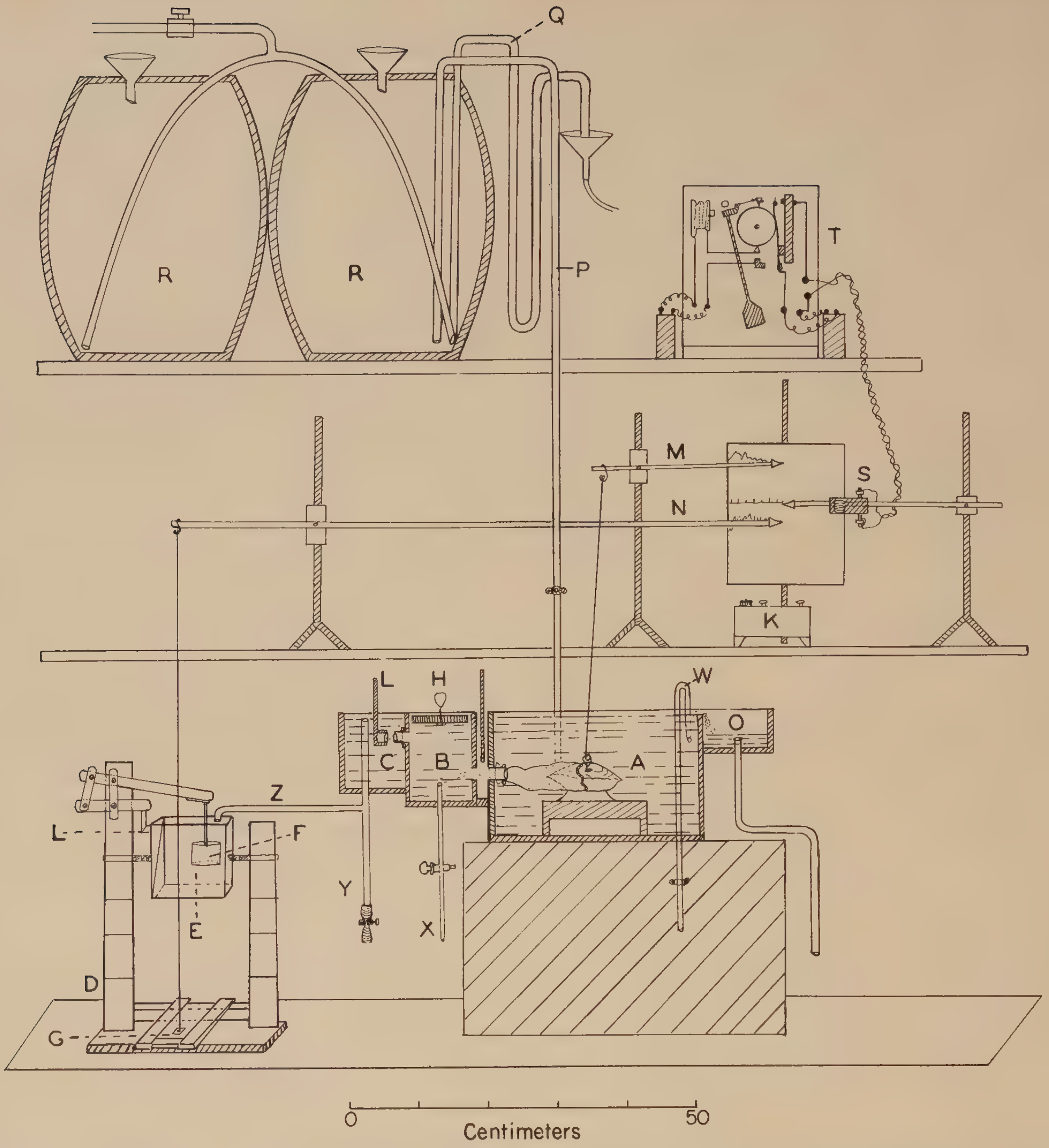

Figure 169.—Diagram of a setup for simultaneous recording of the rate of water transport and shell movements of the oyster. A—vessel with oyster; B and C—small connecting vessels; D—frame of the dumping vessel; E—dumping vessel; F—float; G—springboard to record each overturn of the dumping vessel; H—paraffined float; K—slow motion kymograph; L—cap to close the connection between the two vessel; M—lever recording shell movement; N—lever recording the dumping of water; O—overflow; P—sea-water supply; Q—constant level siphon; R—barrels; S—signal magnet; T—electric time recorder; X, Y, W—tubes for taking samples of water; Z—overflow tube leading to dumping vessel. Temperature recorder is not shown.

intervals. The supply of sea water is delivered to the barrels R set on top of the stand, and the overflow siphon Q keeps the water in the containers at a constant level and insures a uniform rate of delivery of water to the experimental tank *A*.

The size and shape of the dumping vessel can be modified to suit the purpose of the experiment and to facilitate its operation. The capacity of the dumping vessels made in my laboratory at Woods Hole varied from 55 ml. to 226 ml. The methods described above were successfully used in a number of investigations (Galtsoff, Prytherch, Smith, and Koehring, 1935; Chipman and Galtsoff, 1949a, 1949b).

All dumping vessels require frequent adjustments and become unreliable if used continuously for several days. For long-term observations it is more practical to use a water wheel made with two plastic disks mounted on a horizontal glass rod about ½-inch apart. The space between the disks is divided by radial partitions into a series of triangular compartments. The wheel is placed under the overflow tubing of the small vessel which receives water discharged by the oyster. The compartments are arranged in such a way that when they are full of water the wheel turns slightly and the next empty compartment moves into position under the pipe. The wheel is kept half submerged in sea water to prevent spinning. Under this condition the rotation proceeds in smooth steps; when the wheel makes one complete turn the little bar attached to its side touches a string which moves the writing lever and makes a vertical stroke on a slow moving drum of the kymograph. The construction of the wheel (*F*) and the arrangement of different parts of the set in which it was used are shown in figure 170. The wheel is calibrated by measuring the volume of sea water needed to make it turn one complete revolution. The test is repeated at least 10 times, and the average value is taken as the true capacity of the wheel. Wheels of different dimensions may be used. In my experiments I used wheels of about 50- and 100-ml. capacities; the readings were accurate within ±2.5 percent.

The setup shown in figure 170 is specifically designed for studying the effects of various contaminants that may be added at a known rate to the water supplied to tank E. Sea water from the laboratory supply pipe C is delivered to three 5-gal. carboys from which it runs into two tempering jars with electric heaters (group B) and vessel C. The water then passes into mixing chamber D to which the solution to be tested may be added from the two flasks O and N, which contain known concentrations of chemicals or a desired dilution of a culture of microorganisms. Test solutions in flasks O and N may be added directly to the gills (as shown in the diagram, flask N) or may be delivered to the mixing chamber D. If the solution is to go into the mixing chamber the siphon from flasks N or O is turned around 180° so that the tip of the delivery pipe is at the right end of the mixing chamber D. This

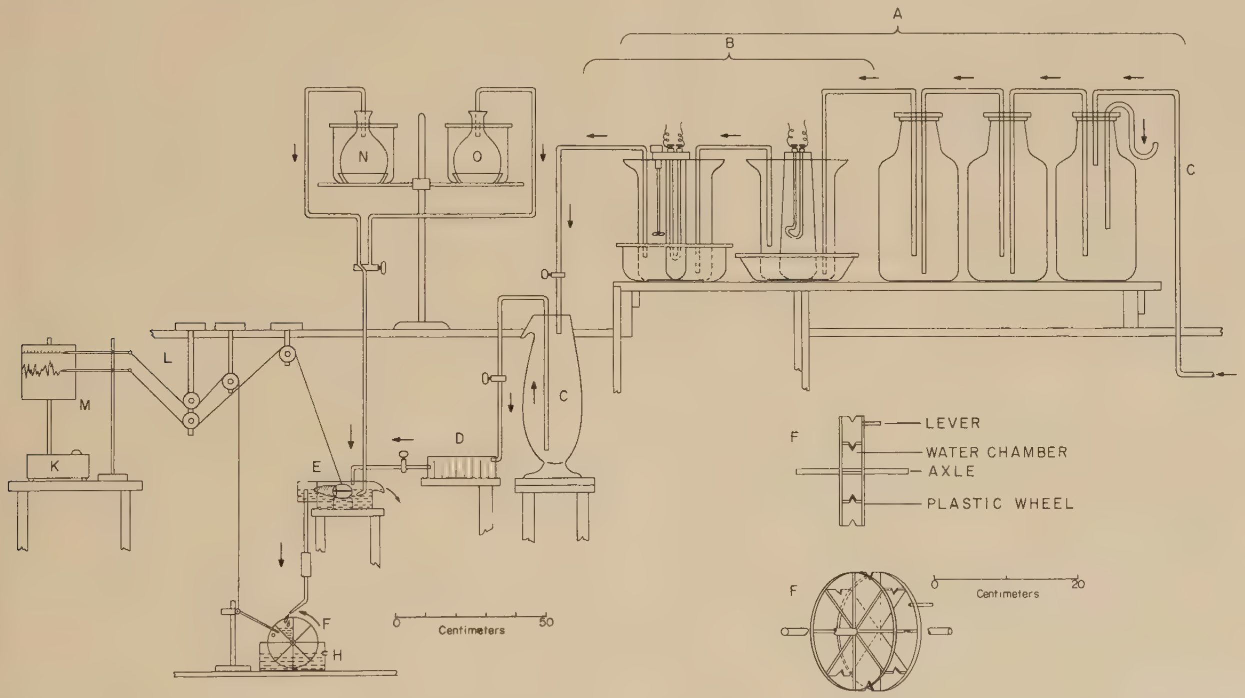

FIGURE 170.—Setup for automatic recording of the amount of water transported by the gills of the oyster. A—series of 5-gal. containers from which sea water is delivered to the tank with the oyster; B—two containers with electric heaters and thermostatic control (not shown in the diagram); C—constant level jar from which water is delivered to mixing chamber D; E—tank with oyster wrapped in apron; F—water wheel; H—container in which the water wheel is partially submerged; K—kymograph; L—writing lever activated by the turning of the water wheel; M—writing lever recording shell movement; N and O—flasks containing solutions or suspensions which may be added either to the mixing chamber or directly to the gills of the oyster.

arrangement was used by Galtsoff and Arcisz (1954) in their work on the effects of known concentrations of *Escherischia coli* on oysters.

For observations intended to last several days or weeks it is convenient to use a slow-motion kymograph, rotating at the speed of about 1 inch per hour. A time marker of 1-hour or ½-hour intervals can be made by mounting a lucite disk on a Telechron type motor making one revolution per hour. A piece of copper wire attached to the periphery of the disk acts as a contact which slides over the two poles of an electric circuit and activates a small signal magnet. Using these methods it was possible to record the ventilation of the gills for 26 consecutive days with only occasional brief interruptions for cleaning the tank and for the removal of feces accumulated inside the apron. An excerpt of such a record is reproduced in figure 171.

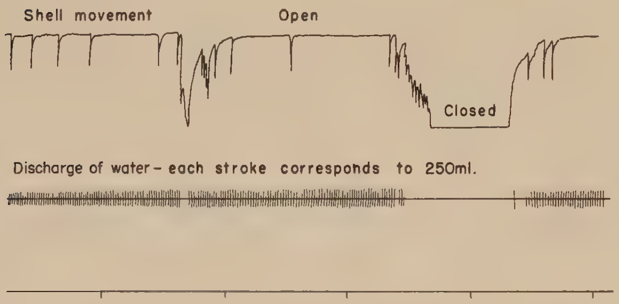

FIGURE 171.—Typical record of the shell movements (upper line) and rate of transport of water by the gills (second line) of an adult *C. virginica* kept in running sea water at the fishery laboratory in Woods Hole at the temperature of 21° to 22° C. Each vertical stroke corresponds to the discharge of a dumping vessel of 250-ml. capacity. Time interval, 1 hour.

An electric drop counter (fig. 172) may be used instead of a dumping vessel or water wheel if the volume of water passed through the gills is small, as for instance in juvenile American oysters or in *Ostrea lurida*. The drop counter is made of a short section of glass tubing with two platinum wires sealed opposite each other. Each time a drop of water falls between the wires the contact is completed and electric current from a transformer or a battery activates the signal magnet and makes a mark on the kymograph drum. The counter works satisfactorily in water of high salinity but is not suitable for brackish water. The number of drops per unit of time is counted from the kymograph record shown in figure 173.

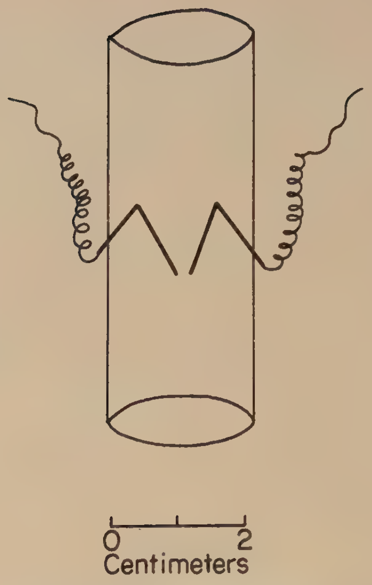

FIGURE 172.—Electric drop counter.

Current indicators.—The relative velocity of gill current can be studied by recording on a kymograph drum the deflections of a shallow cone placed in front of the cloacal opening (Hopkins, 1933). The cone C (fig. 174) about 5 cm. in diameter at its open end is made of lightweight paper waterproofed by dipping in a dilute solution of gum damar in xylene. The cone is mounted on the lower end of vertical rod F[1], which rotates on horizontal axis A and moves the horizontal lever F which indicates the current. The entire system is very light, since lever and rod are made of straw, and it is accurately balanced by connecting the vertical rod F[1] by a hair to a simple

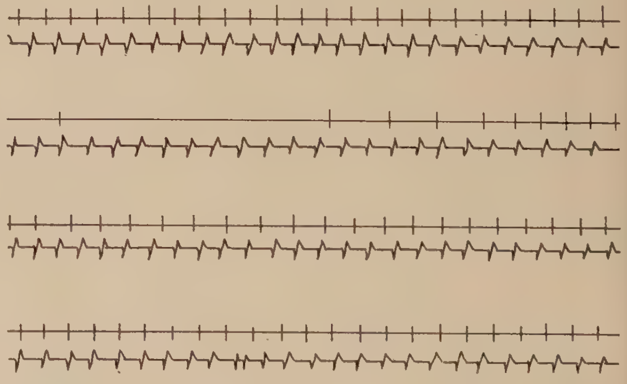

FIGURE 173.—Four parts of a record of the rate of water transport by the gills of a 2-year-old *C. virginica* obtained with an electric drop counter. Upper lines—each stroke corresponds to one drop of water discharged by the oyster; lower lines—time intervals of 1 sec. Temperature of water 22.2° C.

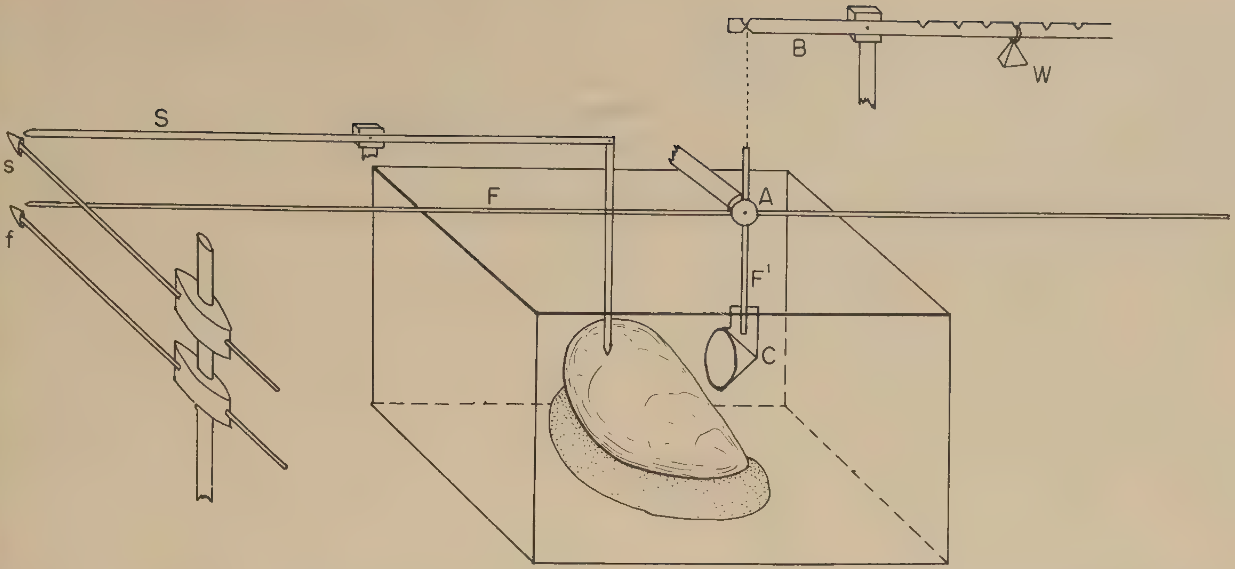

FIGURE 174.—Diagram of apparatus designed by Hopkins (1933, p. 474) to record relative rate of cloacal current and shell movements of oyster. S—lever for recording shell movements; s—fixed bar indicating on kymograph paper closed position of valves; F—lever recording cloacal current; f—fixed bar indicating zero position of F when no current is present; F¹—vertical rod bearing cone C against which the current strikes and turns it on axis A; balance B; adjustable weight—W.

lever B with an adjustable weight W. The cone and current recording lever are fixed to a single stand and so placed that the cone is directly in the path of the cloacal current. The lever S records the movements of the upper valve of an oyster immobilized in cement. The two fixed bars s and f shown at left are set in such a way that they record continuously on the kymograph paper the closed position of the shell (s) and the zero position of current flow (f). The actual amount of water transported by the gill cannot be measured by this method, but the relative values corresponding to the rate of discharge are computed by measuring with a planimeter the area of the record enclosed between the lines made by the excursions of the lever during a known time. A sample of the record obtained by this method is shown in figure 175.

Records of changes in the velocity of the cloacal current obtained in this manner can not be accurately calibrated. In my experience a cone placed in front of the cloacal current frequently fails to come back to the zero position and the entire delicate system easily gets out of adjustment. Another weakness of the method is the uncertainty of the correct position of the cone in relation to the diameter of the stream; it is impossible to know whether the entire width of the column of moving water strikes the cone surface.

A method based on a similar principle was developed by Mironov (1948) in the course of studies of water filtration by Black Sea mussels. The mollusk (M, figure 176) is placed on a horizontal platform (P) mounted on the side of an aquarium about 5 to 6 cm. below the surface of the water. A cover slip (R) freely suspended by two

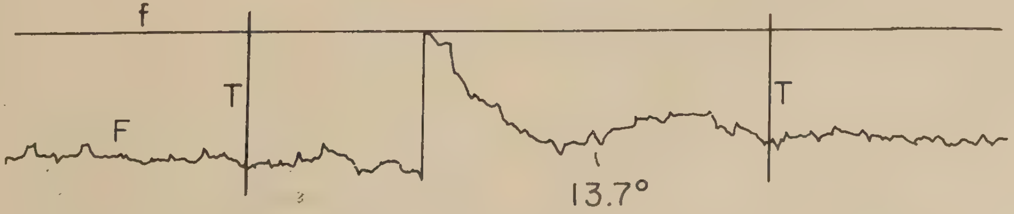

FIGURE 175.—Relative rate of water transport by the gill of *C. gigas* obtained with Hopkins' method at 13.7° C. Reproduced in part from Hopkins' paper, 1933. Portion of kymograph record shows: relative strength of cloacal current F; zero position of lever corresponding to absence of current f; and 5-minute interval on record, two vertical lines T.

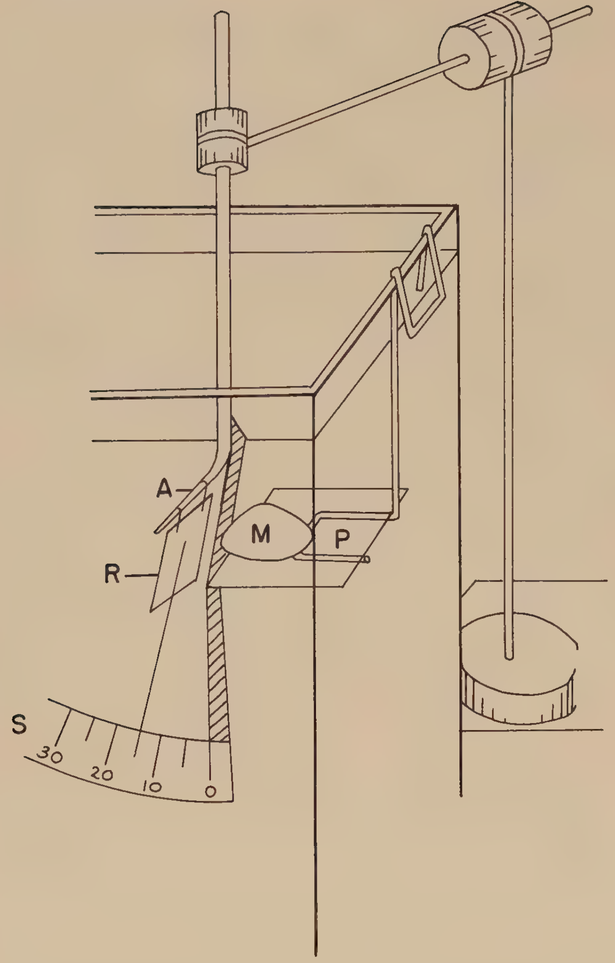

Figure 176.—Mironov's method of recording the rate of water filtration by sea mussel. A—horizontal arm for the suspension of cover slip R; M—mussel; P—platform; S—scale divided in angles. From Mironov, 1948.

glass strings from a horizontal arm (A) of a rod is lowered to such a position that the center of the cover slip is exactly in front of the exhalant siphon of the mussel and is perpendicular to the axis of the cloacal current. A thin glass indicator is cemented by canada balsam to the cover slip; its distal end moves along the scale (S) which is divided in angles. The deflection of the indicator is read every 5 minutes. The apparatus is calibrated by recording the deflection of the glass plate caused by a current of known velocity. For this purpose Mironov used the difference in water levels in the two aquaria A and B shown in figure 177. Water from A runs through an inverted U-tube into tank B. A small inverted T-tube (T) with one end sealed is inserted into the left arm of the siphon (U). The recording assembly of the type shown in figure 177 is placed in front of the cover slip, and the angles of deflection corresponding to the various level differences are read: millimeters on vertical scales L and L_1 placed in each tank. The velocity of the current is computed by using the formula $V=\sqrt{2gH}$ where H is the difference of the heights of water columns in the two connecting tanks and g is acceleration due to gravity. The volume is calculated by multiplying the current velocity V by the cross-sectional area of the opening of the siphon (2.4 mm.^{2} in Mironov's experiments). The average rate of water transport was found to be 15 ml./min., or 0.9 l./hr. Mironov's observations, made within 24 hours, showed great variations in the rate of water transport which undoubtedly indicated that the experimental animals were disturbed and had not reached the steady state.

Lack of automatic registration of the rate of discharge is the obvious deficiency of the method, which could be improved by incorporating a me-

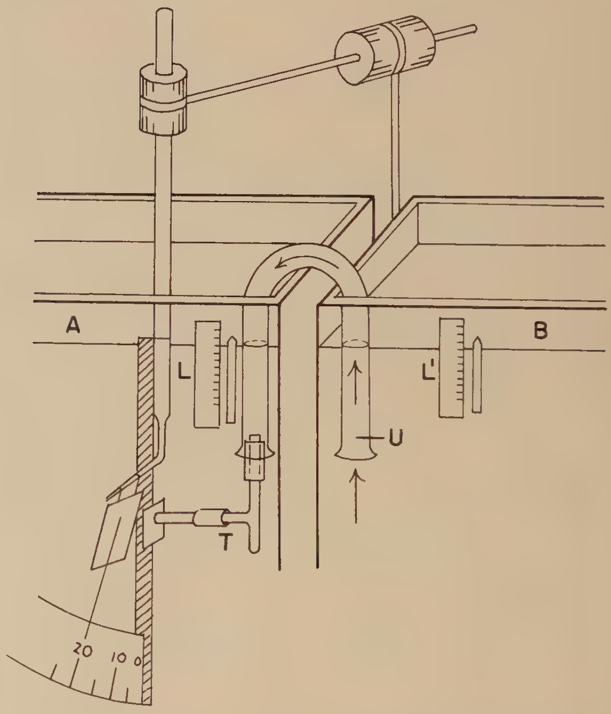

Figure 177.—Mironov's method of calibrating the angle of deflection of a glass plate by the currents of different velocities. A, B—two aquaria tanks; U—inverted tubing acting as a siphon; T—tubing connecting the siphon and the tank A; the horizontal arm of the tubing is placed in front of the swinging glass indicator; L, L_1—scales mounted on side walls of tanks to read the differences in water level.

chanical or optical device for continuous recording of changes in the position of the indicator.

Both Hopkins' and Mironov's methods are of limited value because the mollusks can not be kept in running sea water which would disturb the recording devices. This greatly restricts the application of their technique.

Indirect methods

All indirect methods are based on measurements of the rate of removal of particles suspended in water. Since the volume of water is kept constant, the observations must be completed in a relatively short time in order to avoid the effect of metabolites. This condition seriously limits the usefulness of the methods.

Use of turbid water.—Viallanes (1892) was the first to determine the relative rate of removal of suspended particles by bivalves. He selected a number of small *O. edulis* (18 months old), *C. angulata* of the same age, and *M. edulis* of "an average size" and placed them in separate crystallizing dishes in a tank with running sea water. Dishes of the same dimensions, but without mollusks, served as controls. After several days the sediment that accumulated on the bottom of the dishes was collected, dried, and weighed. He subtracted the quantity of material precipitated mechanically in the controls from the total quantity found in the dishes with mollusks, and assumed that the remainder was proportional to the volumes of water filtered by them. For each liter of water filtered by *O. edulis*, the *C. angulata* filtered 5 l. and the mussel 3 l. Dry clay was added to the experimental tanks in the proportion of 0.0546 g./l. In 24 hours the mussel precipitated 1.768 g. of clay, *C. angulata* 1.075 g. and *O. edulis* 0.199 g. Essentially the same crude method was employed 34 years later by Ranson (1926). He did not record the temperature during the obvervations and made no attempt to observe the shell movements of the mollusks or to note whether the valves remained open all the time during the experiment. In later years the ability of water-filtering bivalves to clear turbid water has been studied by more elaborate methods. The amount of material remaining in suspension has been computed from turbidity observations made by means of a nephelometer (Mironov, 1948) or with an electrophotometer used as a turbidimeter (Lund, 1957). A great variety of suspensions are used in this type of experiment—india ink, colloidal graphite, carmine powder, powdered eggs, ground diatoms, fuller's clay, milk, calcium carbonate, dried mud and others. Mironov (1948) reports that the best material for this purpose is nephelinic grey clay; after washing it gives a very stable suspension in which the precipitation of particles is so slow that it has virtually no effect on experimental results.

In experimental studies of the rate of water propulsion by the California mussel, *Mytilus californianus*, Fox, Sverdrup, and Cunningham (1937) used a suspension of calcium carbonate ($CaCO_3$). The water was stirred continuously to keep in suspension the calcium not removed by the mussels. At frequent intervals analyses were made of the calcium carbonate remaining in suspension and from these data the rate of water propulsion was computed. The rate of precipitation of calcium in the control tanks suggested that in the absence of mussels the amount of calcium suspended in water can be expressed as an exponential function of time and that the amount precipitated in a unit of time is proportional to the total amount which remains in suspension. In the mathematical treatment of the data Fox and his collaborators applied the following exponential equation:

$$p=P_0e-\left(\frac{nm}{M}+a\right)t=P_0e^{-bt}$$

where p is millgrams of suspended calcium per liter; P_0 is the amount of suspended calcium in the closed system at the beginning of the experiment; n the number of mussels in the vessel; m the volume of water (in liters) transported by one mussel in a unit of time; M the total volume of water in the vessel; t the time; e the Napierian base equal to 2.71828; and "A" and "B" the logarithmic decremental constants determined experimentally.

Under the specified conditions of the experiment in a closed system and using the above assumptions, the volume of water transported by one mussel was calculated by the following equation:

$$m=M\frac{b-a}{n}$$

The values obtained for medium sized mussels from 95 to 130 mm. long varied between 2.2 and 2.9 l./hr.

Several drawbacks are common to all methods based on turbidity determinations. The mollusks are kept in a closed system and are subject to abnormal conditions caused by high content of suspended material and accumulation of metabolites. The methods are not suitable for continuous observations since they should be completed in the relatively short time before turbidity of the water is changed because of the aggregation and flocculation of suspended particles. Finally, computation by turbidity observations of the volume of water filtered by mollusks is based on the assumption that mechanical precipitation, due to gravity, remains constant. This, however, is not the case. Jørgensen and Goldberg (1953) found that *C. virginica* removes graphite particles from 5 to 10 times faster from a 4-hour old suspension than from a fresh one. This effect is explained by the difference in the size of the particles which in the aged suspension are about 2 to 3 μ in diameter; in the fresh one they are less than 2 μ. By adding small amounts of carmine suspension to the gill of the oyster it is easy to notice that the filtering efficiency of the gills of *C. virginica* and *C. gigas* is not high and that many small particles appear in the cloacal current. Jørgensen (1943) assumed that all particles are removed as the water is filtered through the gills, and computed the rate of water transport m by using the following formula:

$$m=\frac{(\log \text{conc}_0-\log \text{conc}_t)\,M}{\log e\cdot t}$$

where m is the volume of water (in liters) transported in 1 hour; M is the volume of suspension in liters; conc_o and conc_t are the concentrations of cells or particles at the beginning of the observations and after t hours; and e is the Napierian base (2.71828). The formula can not be expected to give accurate results because it is based on two incorrect assumptions: first, that all suspended particles are removed from the water as it is being transported through the gills; second, that the dispersion of particles in the suspension does not change during the duration of the test. Jørgensen's observations showed considerable differences in the properties of new and old suspensions of graphite, and Chipman and Hopkins (1954) demonstrated that the efficiency of the removal of cells changes with time and is not related to cell concentrations. In their experiments the rapid rate of removal of *Nitzschia* or *Chlamydomonas* cells was followed by a decrease in the filtering efficiency of the gills and in the increased return to the suspension of the phytoplankton cells which had passed through the gills.

These difficulties introduce great uncertainty in the studies of the rate of water transport by the gills based on turbidity determinations. Some of the problems may be solved by the use of radioactive plankton.

Use of radioactive plankton.—The advance of radioisotope techniques has made it possible to employ labeled plankton algae for determining their rate of removal by water-filtering mollusks. Chipman and Hopkins (1954) and Chipman (1959) applied this method in a study of the rate of water transport in bay scallops, and Smith (1958) extended their observations to the clam, *Mercenaria* (*Venus*) *mercenaria*. Single species cultures of the diatom *Nitzschia closterium* (56 μ in length) and a species of *Chlamydomonas* (7μ in size) were made radioactive by the incorporation of phosphorus, P^{32}. The cells grown in a culture medium (modified Miquel solution) that contained virtually no phosphorus except the P^{32}, were highly radioactive. This isotope, emitting only beta particles of rather high energy, was found to be useful for this purpose. The details of the method developed in the Biological Laboratory of the Bureau of Commercial Fisheries at Beaufort, N.C., are described in a paper by Rice (1953). The method is extraordinarily sensitive and allows detection of very slight changes in cell numbers which otherwise would have remained unnoticed. The use of radioactive plankton presents several advantages in studies of the functions of the gills; it allows observations without undue increases in the concentration of suspended material and it makes possible recordings of changes in the rate of water transport which could not be detected with other methods.

CONTROL OF RATE OF WATER TRANSPORT THROUGH THE GILLS

Estimates of the rate of water transport by an adult oyster, made by investigators who have studied the problem carefully, vary from several liters to a maximum of 34 l./hr. (Loosanoff and Nomejko, 1946). Naturally the rate of water transport depends on the size of the oyster, its physiological state, and environment. The absolute figures are, therefore, of little significance unless they are accompanied by data on temperature, conditions under which the tests were made, and size of the oysters used.

It is self-evident that the quantity of water propelled by the gills must depend on the size of the mollusk. No comparative data of this nature are available for the oyster, but determinations by Chipman (1955) and Chipman and Hopkins (1954) of the rate of propulsion of water by the bay scallop (*Pecten* (*Aequipecten*) *irradians* Lamark) clearly indicate this relationship. The data plotted in figure 178 were taken from the table of observations by these authors, who used a suspension of radioactive *Nitzschia closterium* and *Chlamydomonas*. A similar relationship was reported to exist in the California mussel in which the absolute rate of water transport through the gill was found to be a function of the weight of the soft parts of the mollusk (Rao, 1953). The relationship was well defined at temperatures of 20° and 16°, but was indefinite at 9° C. Rao also referred to the activity of mussels from various geographical regions. He stated that, regardless of temperature, mussels from higher latitudes transport water at a greater rate than mussels taken from the lower latitudes. The observations were of brief duration, lasting from 1 to 3 hours and therefore can not be considered as representative of typical behavior of bivalves over longer periods of time.

In the Bureau's shellfish laboratory at Woods Hole records taken continuously for several weeks show considerable variability among oysters of equal size and origin that are kept under identical conditions. These changes in the rate of water transport can not be correlated with changes in the environment. In the tests only large oysters (10 to 12 cm. in height and 6 to 8 cm. in length) were used. They were healthy, free of boring sponge, *Polydora*, and other commensals. Daily fluctuations of temperature did not exceed 1° to 2° C. and salinity changes were less than 0.1 °/₀₀. The range of daily fluctuations in the rate of ventilation by a single specimen varied from 9.9 to 24.3 l./hr. in 1 day, and from 1.1 to 24.3 l./hr. 2 days later. The total quantity of water transported daily by this oyster in the 2 consecutive days of recording was 77.5 and 457 l. In the other oyster tested within the same month of July the range of daily fluctuations in the rate of water transport varied from 0.28 to 3.31 l./hr. in one day to 5.0 to 13.0 l./hr. the following day. The total quantity of water transported in these 2 days was 8.6 and 239 l./day respectively.

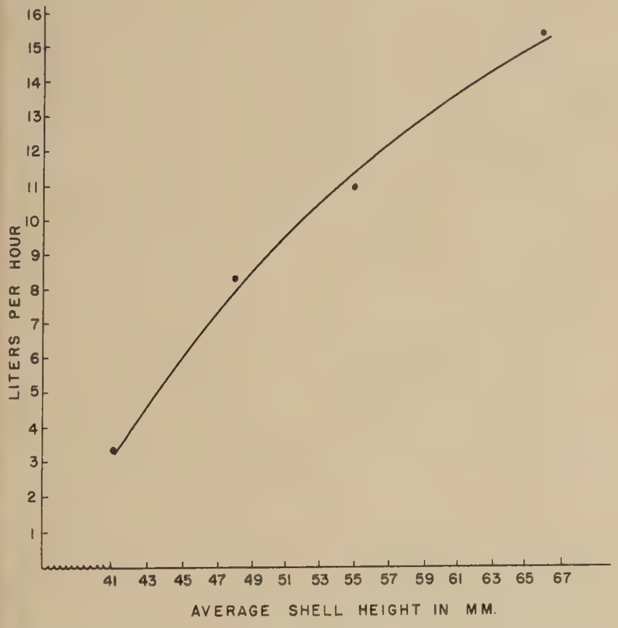

FIGURE 178.—Mean rate of water transport of scallops (*Aequipecten irradians*) of different shell length kept in sea water at room temperature ranging from 21.9° to 25.8° C. The plotted values represent the averages of 6 to 11 specimens. (From the data of Chipman and Hopkins, 1954).

The more than 2,000 records of daily activities of oysters accumulated in the course of many years of my studies confirm this great variation. Some of the records were made continuously for 33 days, others were interrupted after 2 to 3 days of observations. All the records were obtained using the technique shown in figures 169 and 170.

STEADY STATE

Ventilation of the gills may continue for hours without interruption or significant changes in the rate of water transport. This condition, which may be called a steady state, occurs when temperature, salinity, and food content of the water remain constant and the oysters are not disturbed by sudden changes in illumination, vibrations, or other mechanical stimuli. The heart rhythm during the steady state remains constant. Judging by the rate of formation of fecal ribbons, the ingestion of food during these periods continues without interruption, provided the water does not contain excessive amounts of detritus, clay, or plankton which may stimulate the formation of pseudofeces and cause frequent snapping of the valves. The temperature at which the steady state was observed ranged from 15° to 25° C. It is conceivable, however, that it takes place at other temperatures.

An example of the steady state in oysters is shown in figure 179. In this experiment two oysters of approximately the same size were observed simultaneously. Their activities were slightly

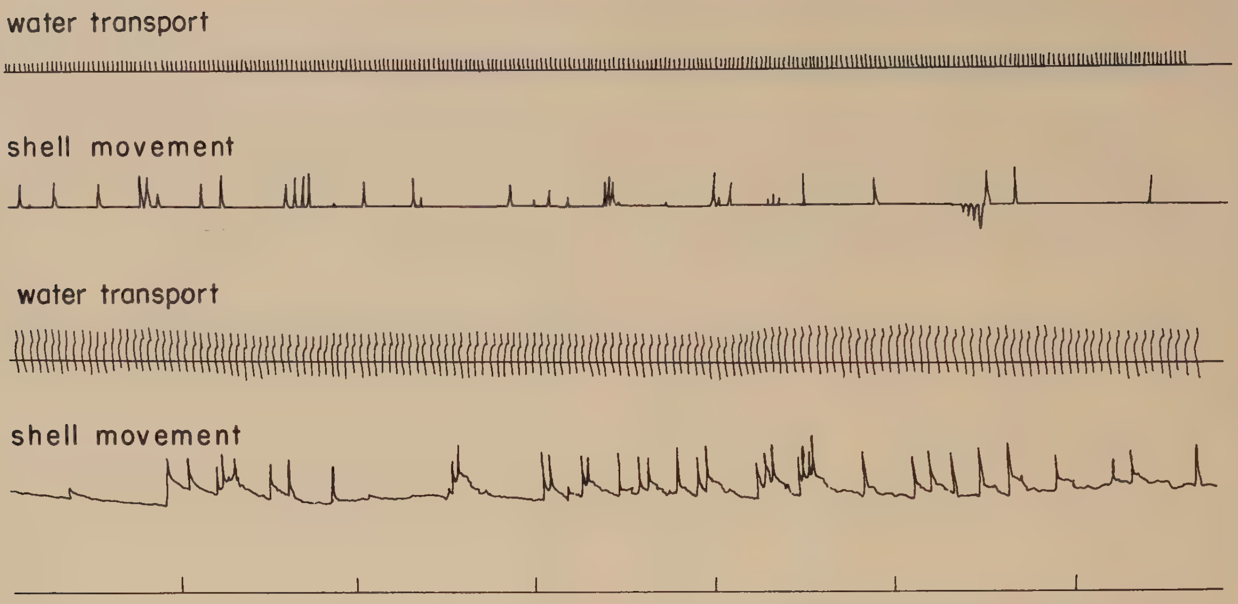

FIGURE 179.—Kymograph tracings of water transport and shell movements of two adult oysters. Each vertical stroke of the first and third line represents the discharge of 57 ml. of water (the capacity of the dumping vessel). Temperature 19.5° C. Salinity 31.2 °/₀₀. Oyster dimensions: 10.6 x 6.5 cm. (upper); 10.5 x 6.6 cm. (lower). Time interval, 1 hour.

different, although both were kept under identical conditions; water was delivered at a uniform rate from a common supply tank, and the temperature was kept constant at 19.5°±0.1° C. The upper record shows that during the 8 hours of observations the rate of water transport of one oyster was fairly uniform, varying between 19 and 21 l./hr. Occasional contractions of the adductor muscle were followed by an immediate return to the former tonus level. In the second oyster (lower part of figure 179) the rate of water transport decreased slightly from 14 l. per hour at the start to 11 l./hr. at the end of the record; its shell movements were more frequent and less regular than in the first oyster. It is apparent from these and other observations of the same type that under identical conditions of environment the rate of water transport may be different, depending on the intrinsic state of the organism.

Water transport by the gills is not carried on with a machine-like performance controlled entirely by such environmental factors as temperature, salinity, chemical composition of water, etc.; it is adjusted to or governed by the needs of the mollusk. It may be assumed that the variable daily requirements for food and oxygen and the necessity of eliminating the metabolites determine both the duration of the activity and the rate of water transport. In addition, the rate of water transport is affected by irregularity of gill activity shortly after spawning during the summer. Oysters often maintain a nearly constant rate of water propulsion for 2 or 3 days. Invariably these periods are followed by periods of partial or total inactivity (closed valves) manifested in greatly reduced rates of water transport or its complete cessation. It appears reasonable to deduce that the needs for food, elimination of products of metabolism, and requirements for oxygen determine both the number of hours per day the oysters stay open and the rate at which water is being transported through the gills.

REDUCTION OF WATER TRANSPORT

Increased and irregular shell movements are usually associated with a decreased rate of water transport. This relationship is apparent in tracings which were made shortly before the closing of the shells or immediately after their opening. The records of three oysters kept at a temperature of about 20° to 22° C. (fig. 180) show that in all of them the rate of water transport slowed down before the valves began to close. Complete closure of the valves took place in 72, 15, and 18 minutes after cessation of the current. In the majority of cases examined the time intervals between the resumption of the cloacal current to

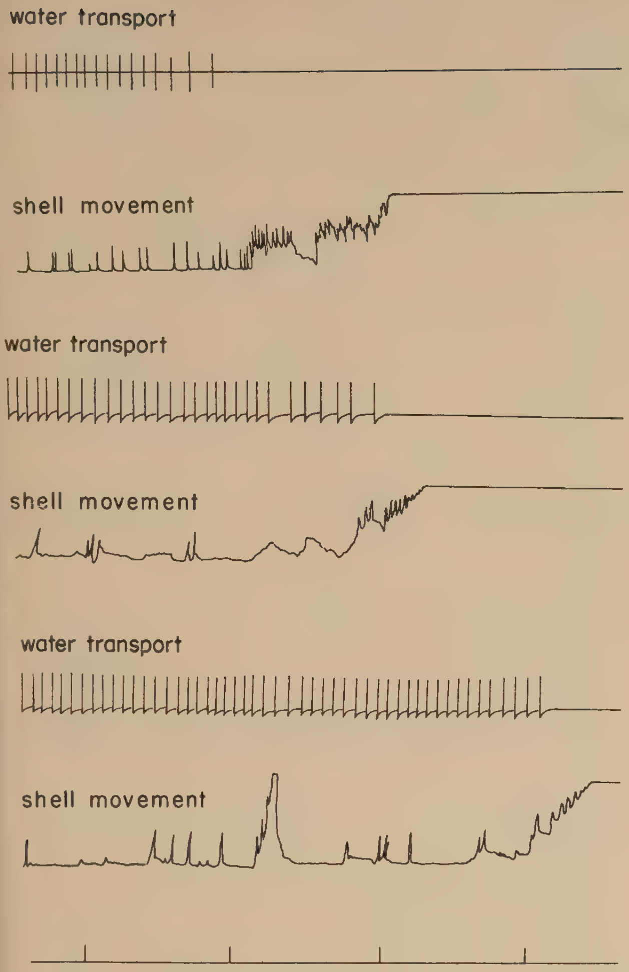

Figure 180.—Tracings of the rate of water transport and shell movements of three adult oysters, *C. virginica* at Woods Hole. Temperature 20° to 22° C. July. Vertical strokes of water transport line correspond to the discharge of 247 ml. of water by the dumping vessel. Time interval, 1 hour.

full velocity and the opening of the valves were about equal (fig. 181).

A reduction in the rate of water transport may also be caused by the slowing down of the lateral cilia of the gill filaments. To study the activity of these cilia it is necessary to eliminate the effects caused by the movements of the shell and mantle. A record of the activity of the lateral cilia can be obtained with an electric drop counter placed under the small tank (fig. 168) to receive water discharged through the cloaca. Parts of such a record made in the summer at Woods Hole are reproduced in figure 173. Interruption of a steady state shown on the second pair of lines in figure 173 was caused by slight tapping against the side of the experimental tank. The lateral cilia are very sensitive to minor mechanical stimuli and slow down at the slightest disturbance. These interruptions were of brief duration, and the preceding rate was restored within a few seconds (line three).

By using the drop counting technique it is possible to observe minor fluctuations in the activity of the lateral cilia and to demonstrate their responses to changes in temperature, salinity, different concentrations of drugs, food particles, etc. In a graphic summary of one such record (fig. 182) the average number of drops per 5 sec. was plotted against time shown at 5-sec. intervals. The rate of current remained fairly constant with the exception of the period between 110th and 145th sec. when a suspension of eggs was added to the gills. The reduction was temporary, and the normal rate was soon resumed.

The dilation and constriction of the ostia and the expansion and folding of the gill lamellae also affect the rate of water transport. The effect of these minor adjustments can not be measured separately from the activity of the lateral cilia. I have noticed, however, that changes in the position of the gill lamellae and the expansion or constriction of the ostia are usually associated with the muscular activities of the adductor muscle and the mantle.

DISSOLVED ORGANIC SUBSTANCES AND RATE OF WATER TRANSPORT

Collier and his associates (1950, 1953) reported that sea water of the Gulf of Mexico contains certain organic substances which have the general chemical chracteristics of carbohydrates. These substances respond to analytical tests with N-ethyl-carbazole (or with anthrone, Lewis and Rakestraw, 1955) and were reported to occur in concentrations up to 50 mg./l. The figure greatly exceeds the concentrations found in sea water by others. According to the reliable studies of Krough (1934) the content of dissolved organic matter in sea water remains fairly constant at the level of about 5 mg./l. It was further claimed by Collier that the carbohydrates occur in variable concentrations in coastal water near Pensacola, Fla., and that their presence greatly influences the shell movements and the rate of water transport by *C. virginica*. Since in their experiments the response of the oysters was not consistent with the concentrations of carbohydrates determined by carbazole reagent, the authors tried to overcome

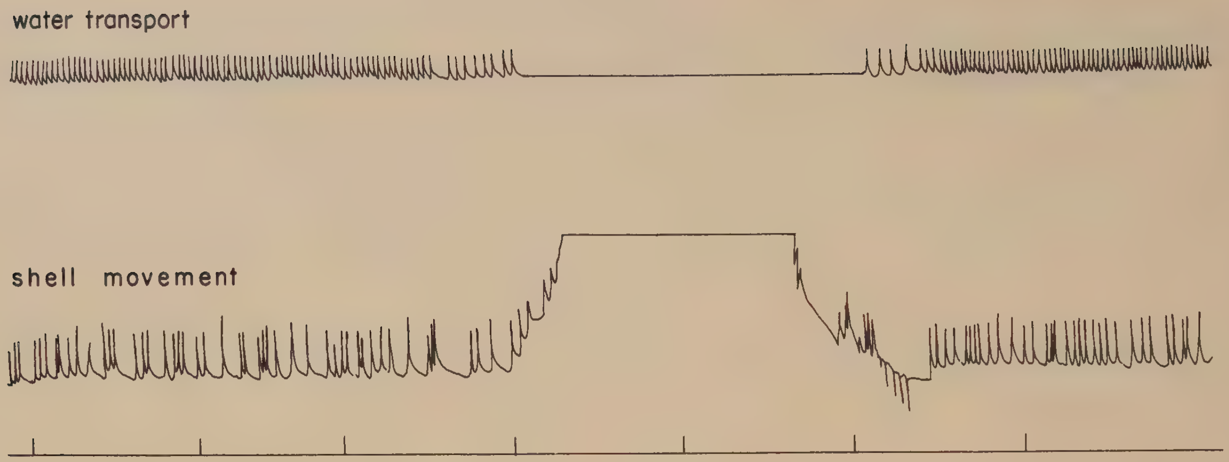

FIGURE 181.—The shell movements and rate of water transport of an adult *C. virginica* preceding the closing (left side) and following the opening (right side) of the valves. Temperature of water 22.5° C. Time interval 1 hour.

the difficulty by assuming that "each oyster appears to have a threshold limit to the carbohydrate below which it will not pump."

The existence of a general chemical factor which controls the principal activity of the oyster would be of great importance to the study of feeding and nutrition of marine invertebrates. It seems that if such a factor actually exists then Putter's theory of the significance of dissolved organic substances in the feeding of marine invertebrates should be reinvestigated, especially in view of the assertion made by Collier and his colleagues that "the oysters remove variable quantities (up to 50 mg./l.hr.) of the carbohydrates from sea water."

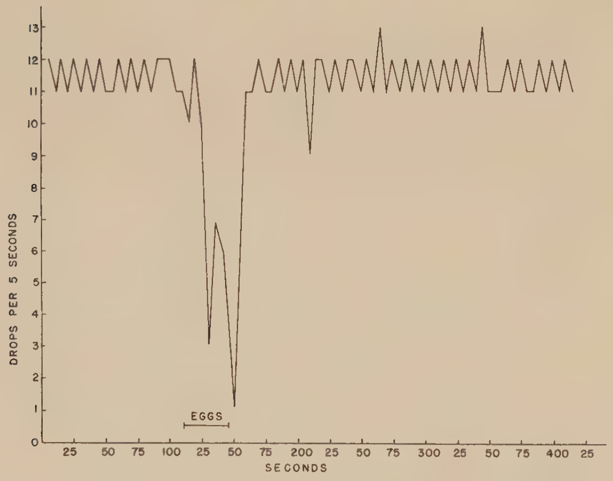

FIGURE 182.—Effects of egg suspension on the water transport by the lateral cilia of the gill of a ripe male oyster, *C. virginica*. Data from the drop counting record are plotted as averages of 5 sec. intervals. Temperature of water 22.5° C.

An attempt to identify the substances concerned was made by Wangersky (1952), who reported the isolation of a compound having the absorption spectrum of dehydroascorbic acid and the presence of a substance which "gives some indication of rhamnoside" and is found in the inshore waters of the Gulf of Mexico in concentrations up to 0.1 g./l.

A description of the reagents and methods for carbohydrate determination can be found in the paper of Lewis and Rakestraw (1955) who remark that in general, the N-ethyl-carbazole method (used by Collier, Ray, Magnitzky, and Bell) is "considerably less satisfactory" than the anthrone method.

The N-ethyl-carbazole method is as follows: 1 g. of the reagent recrystallized from ethanol and water is dissolved in one liter of 90 percent sulfuric acid cooled in ice. The acid should be of highest purity, stored in glass-stoppered bottles. The solution is stored in a dark bottle and kept refrigerated. Exposure to air and sunlight must be avoided as much as possible. Under these conditions the reagent is stable for at least 2 days.

A 2.5-ml. sample of filtered (or centrifuged) sea water is transferred to a 60-ml. bottle, 22.5 ml. of N-ethyl-carbazole reagent is added, and the sample thoroughly mixed. The sample is immediately placed in a water bath at 70° C. (± 0.2° C.) and left for exactly 30 minutes. After 15 to 20 minutes in a refrigerator the sample is allowed to come to room temperature. The optical density at 562 $m\mu$ is determined between 30 to 60 minutes after the removal of the sample from the water bath.

Standard curves and reagent blanks are determined daily with double distilled water. The reddish-violet reaction product is unstable. It is easily destroyed by sunlight and oxidizing agents with a resultant dark green coloration. In filtering the sample it is necessary to establish that passage through the filter does not introduce extraneous carbohydrates.

Collier, Ray, Magnitzky, and Bell (1953), expressed the results of their determinations in terms of concentrations of arabinose (in mg./l.) although the substance or substances present in the water were not definitely identified. They state that they "may not be true carbohydrates."

A series of analyses using the N-ethyl-carbazole method were made at Woods Hole of samples of sea water pumped from the harbor to the laboratory. In July 1953, the carbohydrate content was low, varying from 0.3 to 1.8 mg./l. Many determinations giving values of about 0.1 mg./l. were almost below the threshold of sensitivity of the method and many others had to be discarded because of contamination of the samples or deterioration of the reagent.

A study of the records of shell movements and water transport of oysters in relation to the natural fluctuation of the carbohydrate content of sea water in which they were kept gave negative results. No evidence was found to support the view that the opening of the valves and the resumption of water transport were associated with the increase of carbohydrate concentration from 0.3 to 1.8 mg./l. The oysters opened and closed spontaneously regardless of the slight fluctuations in the content of the substances which react with carbazole. Several excerpts from the laboratory records presented in table 20 illustrate this point. Within the range of concentrations found in the water, the high and low degrees of activity were not correlated with the changes in the carbohydrate content (compare the behavior of oysters B, B^{1} and C, D^{1}). In each case the recording was continued for a period varying from 6 to 8 hours. After periods of inactivity the oysters B^{1}, D, and D^{1} opened and resumed water transport, while there was no change in the concentration of carbohydrate. I deduce from these observations, which were repeated several times with similar results, that the presence of carbohydrates within the range found naturally in Woods Hole sea water had no effect on the oysters and is not a factor which controls the function of their gills.

TABLE 20.—*Carbohydrate concentration in sea water (mg./l.), shell movements and water transport in* C. virginica *at Woods Hole*

[Active shell movement means frequent opening and closing of the valves]

Oyster No.	Temperature	"Carbohydrate"	Shell movement	Water transport
	° C.	*mg./l.*		*l./hr.*
A	16.5	1.3	Active	0
A^{1}	16.5	1.1	Open	5.0
B	15.6	1.8	Active	12.0
B^{1}	16.0	1.8	Partly closed	0
C	21.5	0.8	Active	15.0
D	21.5	1.0	Closed	0
D^{1}	21.5	0.6	Closed	0

The determinations of the concentration of carbohydrates in Woods Hole water are in agreement with the findings of Lewis and Rakestraw (1955) for the Pacific Coast, where they encountered carbohydrates in quantities varying from 0.1 to 0.4 mg./l. and as high as 8 mg./l. in coastal lagoons.

If carbohydrates did in fact influence the rate of water transport it would be reasonable to expect that the addition of these substances in quantities exceeding their concentration in natural water would produce a measurable effect. Quantities of various carbohydrates were added at a known rate to the mixing chamber from which the water was supplied to the constant level tanks with oysters (Galtsoff and Arcisz, 1954). The following substances were used in concentrations ranging from 10 to 100 mg./l.: arabinose, fructose, dextrose, maltose, and ascorbic acid. The tests were continued for several hours. In all cases there was no evidence that the increase in carbohydrate concentration in any way affected the rate of water transport or shell movements of the oysters.

In some instances (first column, table 21) there was a gradual increase in the activity of the gills which cannot be attributed to the presence of arabinose since the rate of water transport continued to increase for several hours after the return of the oyster to natural sea water.

Similar results were obtained in another set of experiments using arabinose and fructose. The following rates of transport of water were recorded:

Before adding arabinose	9.5 to 11.4 l./hr. during 2 hours
In arabinose solution (65 mg./l.)	7.6 to 13.3 l./hr. during 4 hours
Return to natural sea water	19.0 to 26.6 l./hr. during 3 hours

Table 21.—*Rate of water transport, in liters per hour, of three adult* C. virginica *in natural sea water and in water containing 1-arabinose in the concentrations of 65 mg., 80 mg., and 100 mg./l. Salinity 31.2 °/oo*

[In all three experiments the shift from natural sea water to arabinose solution in sea water and again to sea water was made at the end of the hour shown in the first column without disturbing the oyster. Fifteen minutes were needed to flush the experimental tank with natural sea water.]

Time in hours	Water transport in sea water and in arabinose solution in sea water								
	Water transport		Temp.	Water transport		Temp.	Water transport		Temp.
	Sea water	Arab. 65 mg./l.		Sea water	Arab. 80 mg./l.		Sea water	Arab. 100 mg./l.	
	l./hr.	*l./hr.*	° *C.*	*l./hr.*	*l./hr.*	° *C.*	*l./hr.*	*l./hr.*	° *C.*
1	5.7		19.8	3.8		18.2	6.6		17.5
2	4.7		19.8	2.4		18.2	6.6		17.5
3		5.7	19.9		1.6	18.3	0.3		18.0
4		3.8	20.1		1.6	18.3	3.8		18.5
5		6.7	20.2	2.7		18.0	3.8		20.0
6		5.7	20.3	4.1		17.9		5.7	19.8
7		5.7	20.4	4.1		17.9		6.7	20.0
8	9.0		20.8				6.7		20.1
9	11.4		20.9						
10	13.3		21.2						
11	10.4		21.2						

In another test using an oyster with a lower rate of water transport the results were as follows:

Before adding arabinose.....3.9 l./hr. during 2 hours
After addition of arabinose (85 mg./l.).............
4.3 to 4.6 l./hr. during 2 hours
Return to natural sea water.....................
5.6 to 6.0 l./hr. during 2 hours

During both tests the salinity of the water was 31.2 °/oo and the temperature varied between 18.0° and 19.5° C. In the concentration of 102 mg./l. of fructose the following rates were observed:

Before adding fructose........................
3.9 to 4.1 l./hr. during 2 hours
In fructose solution...2.8 to 4.7 l./hr. during 3 hours
Return to natural sea water.....................
4.3 to 4.5 l./hr. during 2 hours

No effects were observed even when the concentrations of carbohydrates were increased to 0.5 percent. The rate of water transport in natural sea water varied between 8.4 and 10.8 l./hr. and from 7.2 to 10.8 in the arabinose solutions. Similar negative results were obtained in 1 percent maltose and 1 percent fructose in sea water and in various concentrations of ascorbic acid.

Additional tests with ascorbic acid were made with the carmine cone method for measuring the velocity of the cloacal current. The results (table 22) show lack of any effect on the efficiency of the lateral cilia in concentrations varying from 10 to 50 mg./l. Velocity of the cloacal current is given in table 22 as an average of 10 consecutive readings made at 2-minute intervals. Fifteen minutes elapsed between each group of 10 readings. The concentration of 400 mg./l. (0.04 percent) completely inhibited the current.

My observations are in full agreement with the results obtained by Butler and Wilson (1959), who presented conclusive evidence that the increases and decreases in the rate of water transport by oysters (*C. virginica*) at the Bureau's Biological Laboratory at Gulf Breeze, Fla., (the place where Collier's experiments were conducted), are not correlated with the changes in the concentrations of carbohydrates in the water and that there is no "minimal threshold level of carbohydrate concentration below which oysters fail to pump."

The experimental studies show that the organic substances which give an N-ethyl-carbazole reaction have no effect on the water transport of oysters in the concentrations in which these substances are encountered in the tidal waters of Cape Cod.

RESPIRATION

Exchange of gases takes place primarily in the gills, but the mantle also has a role of lesser importance in the respiration of bivalves. Observations on the comparative rate of oxygen consumption by various tissues of the oyster have not been made, but the data on oxygen consumption by the gills and mantle of the hard clam *Mercenaria* (*Venus*) *mercenaria* are available. Hopkins (1946) compared the oxygen consumption of the mantle of that species with that of the gills and found that the oxygen uptake by the gills varied from 815 to 912 and that of the mantle only from 11.73 to 15.52 cu. mm./hr./g. of dry

Table 22.—*Effect of ascorbic acid on the velocity of cloacal current of* C. virginica

[Carmine cone method. Temperature 19.6° to 20.6° C. Each figure of velocity is an average of a group of 10 consecutive readings. Time interval between each group—15 minutes.]

Ascorbic acid	Velocity of cloacal current		pH
	In normal sea water	In water with acid	
Mg./l.	*cm./sec*	*cm./sec*	
10	2.1	2.0	8.1
10	2.6	2.9	8.1
10	3.3	3.7	8.1
15	4.3	4.0	8.2
15	2.5	2.6	8.0
15	2.6	2.8	8.0
50	2.1	2.0	8.0
50	3.3	3.7	8.0
400	4.7	no current	8.0

tissue. Similar differences in ratio were observed during all seasons although the absolute figures of oxygen consumption varied. It is probable that a similar ratio may be found in oysters and other bivalves.

METHODS OF STUDY

In the old method of determining the rate of oxygen consumption, oysters were put in a small container filled with sea water which was analyzed for oxygen content at the beginning and at the end of the test. The method was crude since no attention was paid to the increase in the concentration of metabolites in the water or to the opening and closing of the shells during the test period. The results obtained under such conditions were erratic. Some of the defects of this method were eliminated by using the open-chamber technique (Galtsoff and Whipple, 1931). The oyster was mounted on a support and placed in an open jar in sea water under a layer of paraffin oil. Shell movements were recorded on a kymograph. The water in the jar was gently stirred, and the volume drawn off for oxygen determination was replaced by fresh sea water of known oxygen content. The temperature was kept constant by placing the respiratory chamber in a water bath equipped with proper temperature control. The sea water used for the test was filtered to eliminate the effects of photosynthesis and respiration of plankton.

More reliable results were obtained by using the modified respiration chamber of Keys (1930a, 1930b) which was designed originally for studies of oxygen consumption by fishes. The method was used in my laboratory to determine the fluctuations in the rate of oxygen consumption by oysters that were kept for several hours in slowly running water of constant oxygen content (Galtsoff, 1947). During the period of testing the shell movements were recorded and the position of the borders of the mantle was observed. The apparatus (fig. 183) consists of a respiratory chamber A submerged in a large water bath in which the temperature is kept constant within ±0.2°C. Filtered sea water of known oxygen content is supplied by gravity from a battery of carboys, and a uniform rate of delivery is controlled by head pressure kept constant in a small delivery vessel H. The water from the carboys is fed to this vessel at a constant rate; and excess water is voided by suction (on the right side of vessel H) in order to maintain the constant level in H. The tubing that leads from vessel H is divided at I into two branches of equal diameter, one leading to the collecting vessel L, the other to the respiratory chamber A. The constant rate of flow is maintained by means of two capillary glass tubings of equal diameter O inserted in the delivery tube. Stainless steel valves, pinch and glass stopcocks were found unsuitable for this purpose because of the slight shifting of their moving parts. Glass tubings of appropriate diameter were selected for each test from a set of several calibrated capillaries kept on hand, and the rate of flow of water was carefully checked at the beginning and end of each test. Before entering the respiratory chamber A, the water passes through a glass coil B (shown in figure 183 in a vertical position but actually lying flat on the bottom) which is completely submerged in water bath C. The bath is equipped with a constant temperature controller (not shown in figure 183). The water leaves the respiratory chamber through an outlet on the top and runs to one of the collecting cylinders K; by using a three-way stopcock J the flow of water may be shifted from one collecting cylinder to the other. The cylinders are suspended from pulleys and are counterbalanced by weights N. When empty the cylinders are raised above the water tank M; as they are filled with water they descend until they are partially submerged. Heavily paraffined wooden floats prevent direct contact of water collected in the cylinders with the air. For taking samples the cylinder is disconnected from the respiration chamber by turning off the stopcock J so that water is diverted to the second cylinder. Then the closed cylinder may be lifted out and the water taken through the drain cock at the bottom. Glass stoppered Erlenmeyer flasks of 100-ml. capacity were used for sampling. The sample for analysis is taken at the middle level of the cylinder between the 300- and 500-ml. marks. Water which runs directly from the supply carboy is sampled in the same manner. The difference in the oxygen content of the water running in and out of the respiratory chamber multiplied by the rate of flow through the chamber gives the quantity of oxygen consumed by the oyster in a unit of time.

Each oyster was prepared carefully for the tests. Shells were scrubbed with a wire brush, rinsed in fresh water, dried, and covered with melted paraffin applied with a small brush. During this treatment the oyster was kept vertical with its hinge at the bottom, and paraffin was applied in

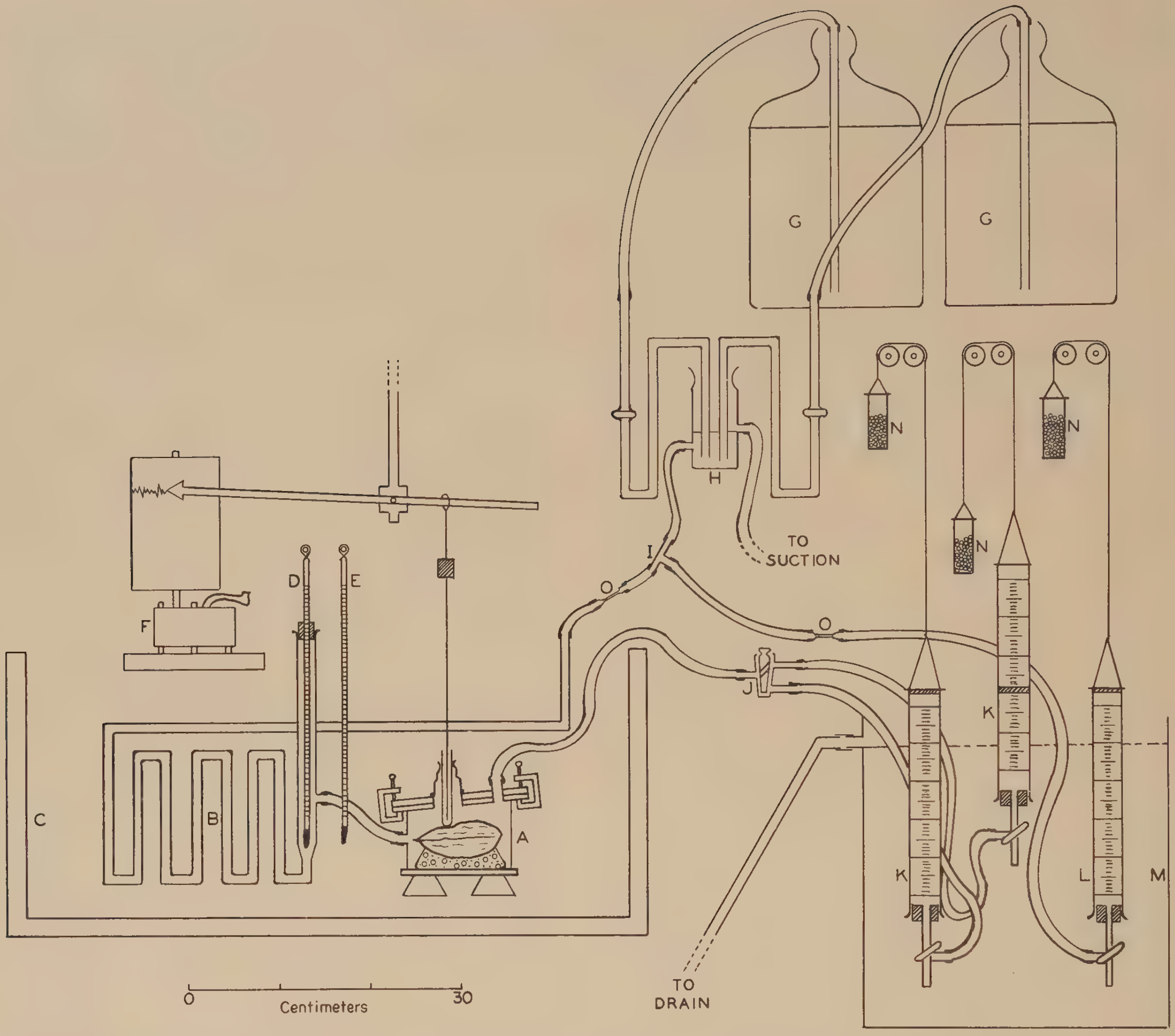

Figure 183.—Metabolism chamber for determining the oxygen uptake of an oyster kept in continuously renewed filtered sea water. A—respiratory chamber (for details see figure 184); B—glass coil (placed horizontally on bottom) to bring the sea water to a desired temperature; C—constant temperature water bath; D—thermometer for recording the temperature of water before it enters the respiratory chamber; E—thermometer for recording the temperature of water in the bath; F—slow-motion kymograph; G—supply of filtered sea water of known oxygen content; H—constant level tank which regulates the flow of sea water through the chamber; I—T-tube connection leading to the sampling cylinder; J—two-way stopcock; K—two sampling cylinders which receive water from the respiratory chamber; L—sampling cylinder which receives sea water directly from supply G; M—water bath in which the sampling cylinders are suspended; N—counter weights to balance the sampling cylinders; O—capillary tubing for regulating the rate of flow of sea water. Constant temperature regulator, heater or cooler in bath C, are not shown.

short strokes directed away from the edge of the shell to avoid accidental sealing of the valves. After careful examination and removal of superfluous paraffin the oyster was placed in the oval respiratory chamber (figure 184), which was built of heavy plastic with a removable slanted top (E) kept in place by two metal clamps (F). The capacity of the chamber, which rests on two heavy lead bars, is about 800 ml. When it is in operation, filtered sea water of known oxygen content is delivered through inlet B and is discharged through outlet C on the top.

The chamber is filled with filtered sea water, the oyster is placed inside, and the cover clamped down. All air bubbles are carefully evacuated. To record the shell movements a glass test tube (H) is lowered through the wide neck of the cover E until it rests on the oyster valve; the

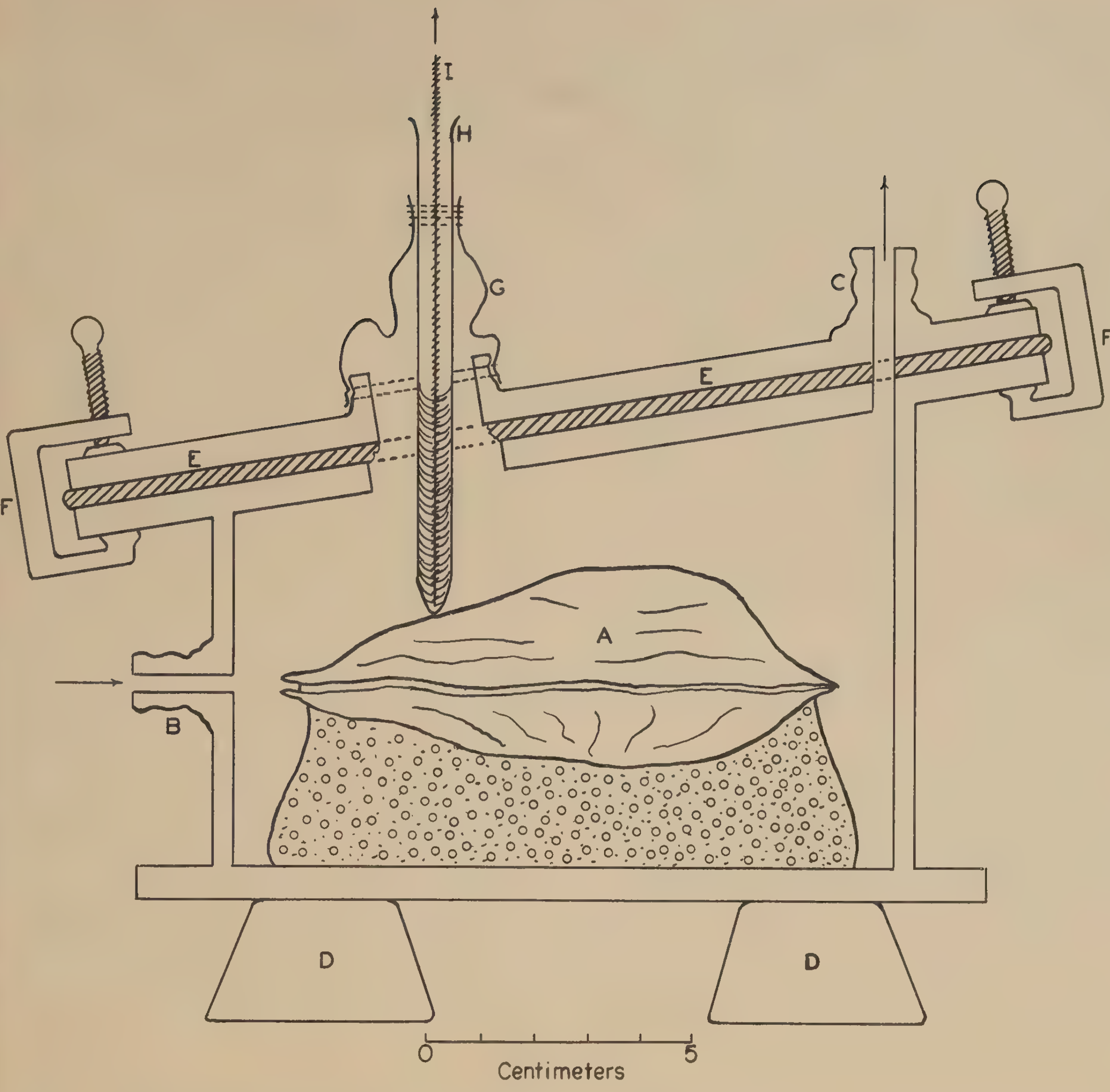

FIGURE 184.—Metabolism chamber, details of construction. A—oyster resting on heavy concrete base ready for metabolism test; B—inlet for sea water of known oxygen content; C—outlet; D—lead bars; E—slanted removable top underlined with a rubber gasket; F—clamps; G—rubber balloon which serves as a flexible gasket for test tube H which rests on the valve of the oyster; I—laboratory brush inserted into the test tube and attached to the writing lever of a kymograph.

small rubber balloon G tied with a silk thread acts as a flexible, watertight gasket with enough slack to permit slight vertical movements of the tube as the oyster opens and closes its valves. The connection with the writing lever of a kymograph is made by inserting a small laboratory brush in the glass tube and attaching the metal handle of the brush to the arm of the lever (fig. 183). Outlet C is connected to the tubing which leads to collecting cylinders K (fig. 183). The chamber without oyster must be checked first by taking simultaneous samples of water from the control and from one of the collecting cylinders. When the samples give identical values of oxygen content a second test is made with the closed oyster in place in the respiratory chamber. If the difference in the two samples exceeds the probable titration error, a search for trouble should be made. Usually it can be traced to a defective paraffin coating of the shell or to the growth of fungi and bacteria inside the rubber connections. To avoid this growth all rubber tubings and joints should be periodically cleaned, dried, and sterilized. With these precautions duplicate determinations in the Bureau's shellfish laboratory gave consistent results, the error not exceeding ±0.01 mg. of oxygen in the 100-ml. sample.

Oysters were kept in the respiratory chamber from 4 to 9 hours, and samples of water were taken at half-hour intervals. The duration of the test was limited by the available quantity of filtered and aerated sea water. For oxygen determination the Winkler titration was used; in some tests the Van Slyke volumetric method was employed for determining the carbon dioxide and the oxygen content of the water.

To obtain data corresponding to the level of basic metabolism, the oysters were starved for 24 hours by placing them in filtered sea water. This period was found to be long enough to cleanse the intestinal tracts and to discard the feces and pseudofeces. Throughout all the tests the temperature of water was maintained at 25° C.±0.1° C.

MICRODETERMINATION OF OXYGEN

Oxygen content in very small volumes of water can be found by using one of the microdetermination methods developed by Lund, 1921; Thompson and Miller, 1928; Kawaguti, 1933; Krogh, 1935b; and Van Dam, 1935a. The volume of water used for analysis in these methods varies from a few ml. to a fraction of 1 ml. Samples can be taken simultaneously or nearly simultaneously from the inhalant and exhalant currents of a bivalve. It is obvious that such a procedure requires great precision of sampling. This is made possible by Krogh's syringe pipette (Krogh and Keys, 1931) or its modification made by Van Dam (1935a). The syringe pipette designed by Krogh and suitable for delivery of small quantities of fluid with a high degree of accuracy is shown in figure 185. It is a glass cylinder with a carefully ground plunger and a heavy-walled glass capillary welded to the tip instead of the conventional metal injection needle of a hypodermic syringe. Tuberculin syringes with blue plungers are suitable for this purpose. The glass capillary is about 5-cm. long with a small bore of inner diameter of about 0.15 to 0.20 mm. The syringe is mounted on a frame of two steel rods set in a bakelite or ebonite base. The notched bar, N, determines the highest position of the plunger. The volume delivered by the syringe is adjusted to any desired fraction of its capacity by a metal collar which may be pushed into one of the notches and set in a fixed position by set screw S. The pipette with the special tip answering Van Dam's specification is not available from stock at any scientific supply store in this country and has to be made to order by an experienced glass blower.

For taking samples two syringes are mounted on an adjustable screw stand that allows fine and independent movement in the vertical and horizontal planes. The syringes are attached to the arms of the stand by ball bearing holders H, so that they can be set at any angle to the horizontal plane. The stand must be heavy and must have adjustments fine enough so that the tips of the collecting syringes can be introduced into the cloacal region of the oyster or the branchial and anal siphons of clams without touching or disturbing the sensitive tissues. The type of stand suitable for this purpose is shown in figure 1 of Van Dam's paper (1935a).

Samples of water for microanalysis also can be taken by means of a siphon; the tip is introduced deep into the cloacal chamber of a bivalve mollusk, as shown diagrammatically in figure 186. This device was used by Van Dam (1954) in his

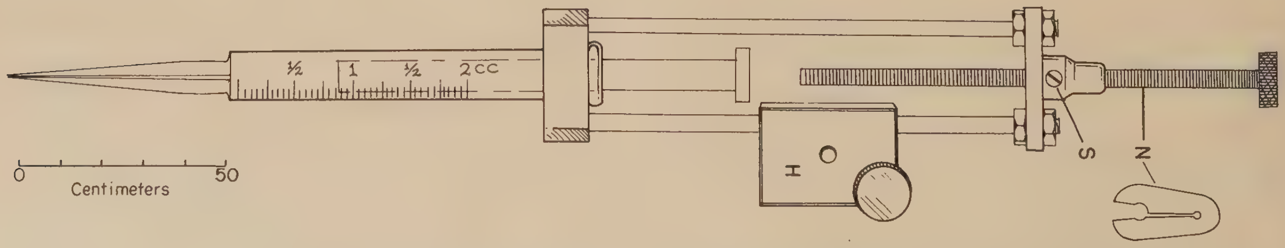

Figure 185.—Van Dam's modification of the syringe pipette for taking small samples of fluid with a high degree of accuracy. H—clamp holder; N—notched bar; S—set screw.

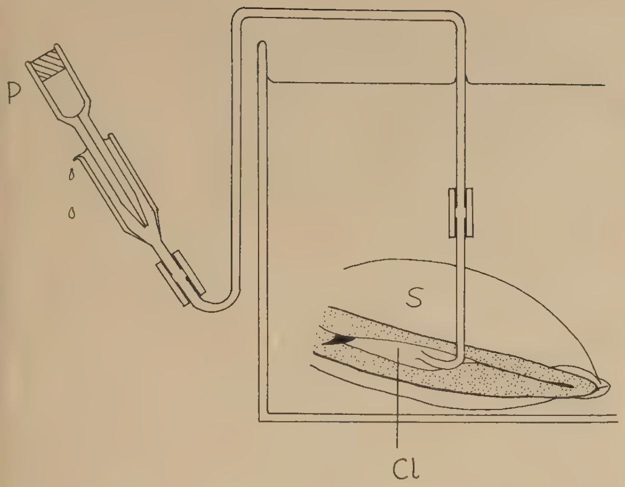

FIGURE 186.—Van Dam's method of sampling sea water from the exhalant current of sea scallop. Samples are taken by syringe pipette P (shown in part, left side) from the end of siphon with the lower part introduced into the cloaca, Cl. The horizontal arm of the siphon is about 5 mm. inside the cloaca. S—scallop. From Van Dam, 1954.

study of respiration of the scallop. The presence of the tip of the siphon in the cloaca does not interfere with the normal propulsion of water, provided the needle does not touch the scallop. Before the first samples were taken Van Dam allowed the water from the cloaca to pass through the siphon for about 1 hour at the rate of 1.5 ml. per minute. Such a slow rate of collecting was considered a guarantee that the sample was not contaminated with outside water. The difference between tne oxygen content of the water that entered the gill and of the water of the exhalant current showed the percentage of utilization of oxygen.

In bivalves with long and narrow siphons, as for instance *Mya* or *Mercenaria*, water leaving the exhalant aperture probably has a uniform content of oxygen. In species lacking siphons the cloaca opens as a wide cone-shaped slot and the stream of water escaping from the cloaca contains considerable and variable amounts of outside water depending on the distance from the epibranchial chamber. This introduces uncertainty in interpreting the results of the test. Van Dam (1954) found that in bay scallops the oxygen content of the samples taken at different positions within the cloacal current varied from 28.5 to 66.6 percent of the oxygen content of the inhaled water. Because of this uncertainty the method does not appear to be suitable for measuring tne true oxygen utilization of bivalves unless the tip of the collecting syringe is introduced deep inside the epibranchial chamber of the gills. Since the rate of propulsion of water and the volume of water transported are not known, the total quantity of oxygen used cannot be determined. This greatly limits the usefulness of the method. The main advantage of microdetermination methods is that the mollusks may be kept in running sea water under conditions which closely approach their normal environment.

The selection of a method for respiratory studies must be governed by the purpose and conditions of the experiments. All closed system methods are suitable for tests that should be completed before the depletion of oxygen begins to affect the respiratory rate. The microanalytical methods are suitable for determining the utilization of oxygen by bivalves (i.e., the percent of oxygen removed by tissues during the transport of water) provided the samples of the exhalant current are not contaminated with outside water. This is nearly impossible to avoid in species like scallops and oysters, which have no siphons. The use of the respiratory chamber with a constant rate of flow of water seems to be the most satisfactory technique for long-term observations on oysters. The method gives reproducible values of the oxygen uptake over a period of many hours. The following discussion of the respiration of the oyster is based primarily on results obtained in the Bureau's shellfish laboratory with this method.

OXYGEN UPTAKE

The rate of oxygen uptake is influenced by several extrinsic and intrinsic factors. The first group includes seasonal and diurnal changes in the temperature and salinity of water, and the occasional presence of contaminants or other environmental changes, such as an abundance of unicellular algae which may depress the rate of respiration. The existence of the intrinsic factors becomes apparent in observations of differing metabolic rates in oysters of known origin and uniform size and age kept under constant conditions (see: p. 207). Some of the intrinsic factors are associated with differences in the contents of water and glycogen in the tissues; with the loss of solids due to the discharge of sex cells during spawning; and with generally poor condition of the oysters. In a comparative study of respiratory rates several precautions are necessary to minimize the extent of individual variations.

Oysters should be taken from a healthy population; they should be devoid of parasites and commensals; they must be of uniform size and age. The metabolism tests should be made at constant temperature and salinity.

Observations described below were made in accordance with these requirements. Oysters obtained from grounds near the laboratory varied from 9.6 to 10.3 cm. in height and from 6.5 to 7.0 cm. in length. They were fully adjusted to the salinity of the laboratory water. The temperature of the water in the respiratory chamber was kept between 24° and 25° C. but changes during each test did not exceed ±0.1° C. The salinity of water was kept constant at the concentration corresponding to the salinity of their natural environment. Tests were performed at Woods Hole and in Milford, Conn.[8]

Shell movements were recorded continuously during tests which lasted from 3.5 to 8.5 hours, depending on the behavior of the oyster. If the oysters remained closed for more than 30 minutes the test was discontinued, since it was reasonable to expect on the basis of previous experience that the period of closure would continue for several hours. Samples of water for oxygen determination were taken at half-hour intervals. For the study of seasonal changes of respiratory rates the oysters were marked by engraving a serial number on their left valves. Between tests they were kept in the harbor or in a large outdoor tank with circulating sea water. The data of oxygen uptake are expressed either as cu. ml. of oxygen (at 0° C. and 710 mm. barometric pressure) or as mg. of oxygen consumed per oyster per hour. (To convert the number of ml. into mg. of oxygen the first value should be multiplied by 1.4292.)

Oxygen uptake of animals is usually expressed per unit of their body weight. In the case of the oyster the use of the total weight may be misleading because of the great variations in the weight of metabolically inert shell material. It is, therefore, more sensible to refer to the oxygen uptake per unit of either wet or dry tissues. The use of dry weight gives more consistant results because in this way the variability caused by changes in the water content of the tissues is eliminated. The rate of oxygen uptake by a single oyster of known size is, however, of interest to ecologists who are concerned with the oxygen requirements of an entire oyster community. Furthermore, in a study of seasonal metabolic changes the experimental oysters could not be sacrificed at the end of each test. Their weight at the end of the entire series of observations would be meaningless because of the changes in solids. The data on seasonal variations in respiration are, therefore, given in the amounts of oxygen consumed by a single adult oyster in 1 hour.

The range of individual variations in the rate of oxygen uptake by adult oysters of approximately equal size is fairly large. Wide fluctuations frequently occur during a single test until the oyster reaches a steady state and remains open with a minimum of shell movement. Therefore, estimates of oxygen demand based on one or two readings made shortly after placing the mollusk in the respiratory chamber are meaningless. As the observations described below indicate, the study of the respiratory rates should be based on a series of readings continued for several hours and made at regular intervals.

Table 23 presents a summary of observations made on 11 adult Long Island Sound oysters, which prior to the tests were kept for at least 4 weeks in Woods Hole harbor and were adjusted to the salinity of the laboratory water. With a few exceptions the oxygen uptake of each oyster remained fairly constant during the test. The mean oxygen consumption per oyster per hour varied from 3.0 to 5.8 mg. The mean value for the entire group was 4.08 mg. of oxygen per hour per oyster. The group apparently divided into two classes of oysters, those with the low metabolic rate of 2.5 and 3.6 mg. of oxygen per

Table 23.—*Oxygen uptake in mg. per hour per oyster of 11 adult Massachusetts oysters during 3.5 hours at half hour intervals at the temperature of 24° to 25° C.*

[The tests were made at the Woods Hole laboratory in June before the beginning of spawning. Nos. 4, 8, and 11 are females, the others are male. Dimensions of oysters: height 9.6 to 10.3 cm.; length 6.5 to 7.0 cm.]

Hours after start	Oyster Number										
	1	2	3	4	5	6	7	8	9	10	11
0.5	4.5	1.8	2.9	5.1	3.9	1.9	6.3	4.8	3.6	3.7	4.8
1.0	5.4	2.8	2.9	5.0	4.2	2.5	5.7	4.7	3.3	3.8	4.8
1.5	4.3	3.0	2.9	5.2	-----	2.0	5.7	5.0	3.8	3.7	5.1
2.0	4.2	3.2	2.9	5.1	4.0	2.7	5.9	4.8	3.1	3.4	5.3
2.5	4.0	3.7	2.9	5.2	4.1	2.9	5.6	5.1	2.9	3.5	5.7
3.0	3.9	3.8	3.4	5.1	3.9	2.9	5.8	5.2	3.0	3.7	5.4
3.5	3.7	3.8	-----	5.0	3.9	2.8	-----	4.6	3.1	-----	4.9
Mean	4.29	3.16	3.0	5.13	4.01	2.54	5.84	4.89	3.26	3.64	5.17
Std. dev.	±0.56	0.72	0.25	0.07	0.11	0.42	0.26	0.24	0.33	0.16	35.0

Mean of means 4.08.
Standard deviation ± 0.337.

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[8] I gratefully acknowledge the valuable cooperation of Walter A. Chipman in conducting for me a series of tests at Milford; and to two medical students, now doctors, John F. Reppun and George Mishtowt, who assisted me at Woods Hole.

hour, and those in which the mean oxygen consumption was higher varying from 4.0 to 5.8 mg. of oxygen per hour per oyster. These differences could not be associated with sex or sexual maturity. The three females of the group (Nos. 4, 8, and 11) had a high metabolic rate, but an even higher value was recorded for one of the males (No. 7). Subsequent tests showed that all 11 oysters were sexually mature and upon stimulation spawned copiously.

For comparison the metabolic rates of six oysters from Onset, Mass., were determined. The same technique was used but the duration of each observation was extended to 7.5 to 8.5 hours (table 24). The tests were completed during the last week of July and the first week of August. The mean oxygen uptake in these oysters varied from 2.99 to 4.24 mg. per hour per oyster, and the mean value for the entire group was 3.47. After the test was finished the oysters were examined and found to be partially spawned, which suggested that their lower metabolic rate possibly was associated with the loss of sex cells.

To clarify this point three tests were made with 11 adult New England oysters of approximately the same sizes as those of the Onset animals. After the first test, made early in July, the oysters were induced to spawn several times in the laboratory and were tested again 2 weeks and then 1 month after spawning. The data shown in table 25 are the mean values of six consecutive readings.

A significant decrease in the uptake of oxygen 1 month after spawning was found in all these oysters. The difference was less pronounced 2 weeks after spawning, possibly because of the incomplete discharge of sex cells. The rapid decrease in the metabolic rate is shown by plotting the rates of oxygen consumption as percentages of the initial rate observed before spawning (fig. 187). This inference that basal metabolic rate decreases after spawning is substantiated by data on seasonal changes in the composition of oyster meat (ch. XVII) which shows that the lowest

TABLE 24.—*Oxygen uptake in mg. of oxygen per hour per oyster of six adult oysters from Onset, Mass.*

[Tests made between July 12 and August 7 after the completion of spawning. Temperature 25.1° C.]

Hours after start	Oyster Number					
	1[1]	2	3	4[2]	5	6
0.5	4.2	5.4	3.5	4.6	4.2	3.6
1.0	2.2	3.4	3.9	4.3	3.6	2.0
1.5	2.5	3.8	5.7	4.3	3.6	4.8
2.0	1.3	3.6	3.8	4.2	3.4	1.0
2.5	3.2	3.6	6.2	4.2	3.5	2.0
3.0	3.5	3.7	4.5	3.8	3.2	3.1
3.5	3.5	3.9	3.8	4.1	3.9	3.1
4.0	3.8	3.3	3.8	4.0	3.4	3.0
4.5	4.6	3.0	3.9	4.0	3.4	3.4
5.0	3.4	3.5	3.6	2.6	2.1	4.4
5.5	3.4	3.7	4.2	1.5	1.4	3.4
6.0	3.6	3.7	3.8	1.8	1.7	3.4
6.5	3.3	4.0	3.8	1.4	1.3	3.4
7.0	3.8	3.4	4.3	--------	--------	3.0
7.5	3.3	--------	4.2	--------	--------	3.4
8.0	--------	--------	4.0	--------	--------	3.1
8.5	--------	--------	--------	--------	--------	3.1
Mean	3.31	3.71	4.24	3.45	2.99	3.12
Standard deviation±	0.80	0.67	0.76	1.33	0.98	0.75

NOTE: Mean of all observations 3.47±0.48 mg. oxygen per oyster per hour.

[1] Partially closed from 1.5 to 2.0 hours; fully open at 2.5 hours.

[2] Oyster partially closed from 5.0 to 6.5 hours.

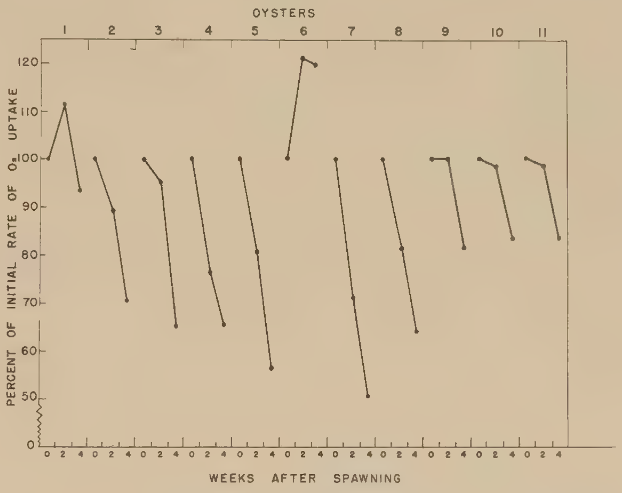

FIGURE 187.—Decrease in the rates of oxygen consumption in 11 adult *C. virginica* from Long Island Sound after spawning. The changes are plotted as percentages of the initial oxygen uptake before spawning.

TABLE 25.—*Mean oxygen uptake, in mg. per hour, per oyster, determined early in July before spawning, at 2 weeks and then 1 month after spawning*

The figures are the mean values of each test and were computed from six consecutive readings made at half hour intervals at temperature 24.0 to 25.0° C.

Time of Observation	Oyster Number												Standard deviation
	1	2	3	4	5	6	7	8	9	10	11	Mean	
Before spawning (July)	6.1	4.8	4.3	7.3	5.7	3.6	8.3	7.0	4.4	5.2	7.4	5.8	±1.51
Two weeks after spawning (August)	6.8	4.3	4.1	5.6	4.6	4.4	5.9	5.7	4.4	4.0	7.3	5.2	±1.14
One month after spawning (August)	5.7	3.4	2.8	4.8	3.2	4.3	4.2	4.5	3.6	3.2	6.2	4.2	±1.08

solid content of oysters occurs shortly after the spawning season. It will be shown later (ch. XIV) that the gonads of sexually mature oysters may constitute as much as 40 percent of the body weight and volume, exclusive of the shell. The loss of a considerable portion of gonad tissues may account for the lower oxygen uptake.

The amount of oxygen consumed by an organism during a unit of time depends on its weight. In the oyster this relationship is obscured by wide fluctuations in the proportion of solids to water. In a series of tests made during the second half of August at Woods Hole oysters of different weights were selected from an oyster bottom at Onset at the head of Buzzards Bay, Mass. The total weight of individual oysters varied from 80 to 203 g. and the wet weight of their tissues ranged from 11.35 to 23.25 g. The oysters had already spawned but still retained a substantial amount of sex cells, with the exception of one oyster in which the gonad was empty and its sex was not recognizable. The data given in table 26 are the mean values of oxygen uptake computed for each oyster from 10 consecutive readings made at half-hour intervals. The rate of oxygen consumption per oyster per hour varied from 3.97 to 7.29 mg. of oxygen. The oxygen consumption of the three heaviest oysters (Nos. 1, 2, and 3) was higher than for the others, but there are no significant differences in oxygen uptake per unit of dry weight according to oyster size (fig. 188). The oxygen demand expressed in this way varied between 2 and 3 mg. per hour.

In the majority of tests the initial oxygen consumption measured within the first hour after placing the animal in the respiration chamber was noticeably higher than in the successive samples. This phenomenon recorded for five out of the total of six oysters (table 24), represents the effect of an oxygen debt incurred during the time the oysters were closed while being prepared for the test. The rate of oxygen uptake usually reaches a more or less stable level after a variable period of adjustment to the new situation. In figure 189 the rate of metabolism recorded continuously for 8.5 hours is plotted against time. Partial closure of the valves was accompanied by a decrease in oxygen consumption (line B). Both oysters A and B reached a steady level of oxygen uptake after the initial periods of adjustment, which in the case of oyster A required 4 hours.

Table 26.—*Oxygen uptake per hour of adult* C. virginica *from Onset, Mass.*

[End of August. Salinity of water 31.2 to 31.3°/oo. Temperature 24.0° to 25.0° C.]

Oyster	Sex	Wet weight	Dry weight	Solids	Oxygen uptake	
					Per oyster	Per 1 g. dry tissue
		G.	*G.*	*Percent*	*Mg./hr.*	*Mg./hr.*
1	?	23.25	2.65	11.4	5.88	2.22
2	M	21.81	3.19	14.6	6.76	2.12
3	F	21.07	2.63	12.5	7.29	2.77
4	M	18.34	2.57	14.0	4.59	1.79
5	M	16.04	2.36	14.7	4.06	1.72
6	M	14.52	1.86	12.8	4.40	2.37
7	M	14.38	2.01	14.0	5.56	2.77
8	M	12.06	2.00	16.5	4.32	2.16
9	M	11.48	1.73	15.1	3.97	2.29
10	M	11.35	1.68	14.8	4.43	2.63

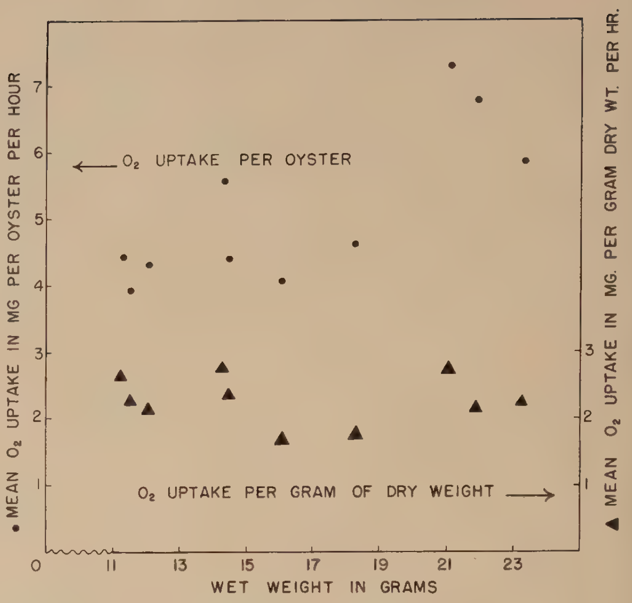

Figure 188.—Mean uptake of oxygen expressed in mg. per oyster (circles) and mg. per 1 g. of dry weight per hour (triangles) in relation to the wet weight of tissues. Each item is a mean value of 6 to 10 consecutive determinations of oxygen consumption of a single oyster. Temperature 24.5° C.

The rate of oxygen uptake decreases with a partial closing of the valves, presumably because of the decrease in the rate of water transport. In the test summarized in figure 190 the two oysters A and B remained in a steady state for nearly 4½ hours. One of them (oyster A, solid line) then began to reduce the opening between the valves and completely closed them at 7½ hours. The decline in oxygen consumption corresponded to the shell movement and registered zero at the moment the shell was closed. No measurable changes in oxygen content in the water were noted after the valves remained tightly closed for some time.

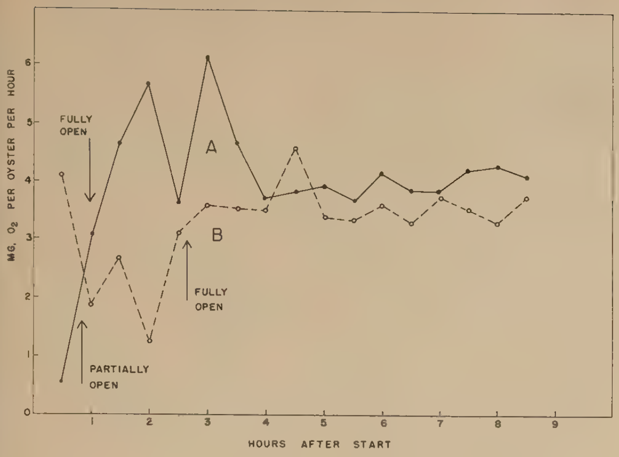

FIGURE 189.—Oxygen uptake, in mg. of oxygen per oyster, per hour, measured at half-hour intervals. Temperature 24.5° C.; salinity of water 31.9°/₀₀. Oyster A (solid line): wet weight of tissues 16.1 g.; dry weight 2.5 g. Oyster B (broken line): wet weight of tissues 12.5 g.; dry weight 1.4 g. Before the tests were made these 6-year-old oysters from Long Island Sound were kept for 35 days in Woods Hole Harbor. August.

Oxygen Uptake Under Continuous Pull of the Adductor Muscle

Increased shell movements of oysters are associated with an increased oxygen uptake. This has been reported by Galtsoff and Whipple (1931), who found that oxygen consumption by the oysters which made only five or less shell closures per hour was about 20 percent lower than that of oysters which closed and opened their valves 30 times or more per hour. The effect on the metabolic rate of the oyster of the resistance of the adductor muscle to a continuous pull and of the maintenance of a forced muscle tonus has been studied for large New England oysters using the equipment designed to determine the power of the adductor muscle (see: p. 176, ch. VIII). The entire platform upon which the oyster was mounted was lowered into a tank of 3 l. capacity filled with filtered sea water and covered by a layer of light mineral oil about 0.5 inch thick. The water was stirred gently, and the temperature was kept constant at 24°±0.3° C. Samples of water, of 100-ml. capacity, withdrawn from the tank were immediately replaced by an equivalent volume of filtered sea water of known oxygen tension. An accurate record was kept of the total volume of water in the tank, and its oxygen content was recomputed after every addition. During the tests, which lasted from 3 to 6 hours, sampling was made every 30 minutes. The initial oxygen tension varied in different experiments from 4 to 5 ml. per l. As the muscle was stretched by the pulling force of 4 kg. (fig. 191), the oxygen uptake, which ordinarily was 3.7 to 5.0 mg. per hour per oyster, decreased to 2.2 mg. Similar results were obtained in several tests in which the pulling force varied from 2 to 4 kg. per oyster. In all cases the adductor muscle was stretched but continued to maintain tonus at a new level. In many instances the up and down shell movements persisted but on a noticeably reduced scale. In the majority of cases the oxygen uptake decreased markedly to almost zero as the shell movements were reduced. During the test shown in figure 192 the shell movements were limited and their character did not change after the pulling force of 2 kg. was applied. The new tonus level was accompanied by an immediate decrease in the uptake of oxygen to about one-half its preceding rate. In 2 hours the consumption of oxygen almost stopped.

In several instances a sudden increase in oxygen consumption was observed when the oyster was in the respiratory chamber despite a pulling force of 2 kg. (fig. 193). Examination of the water revealed the presence of eggs or sperm released from the gonad. The metabolic rate of the sex cells contained in the gonads was suddenly increased as soon as the cells were free in water. Within the gonad tubules the sex cells are tightly packed and

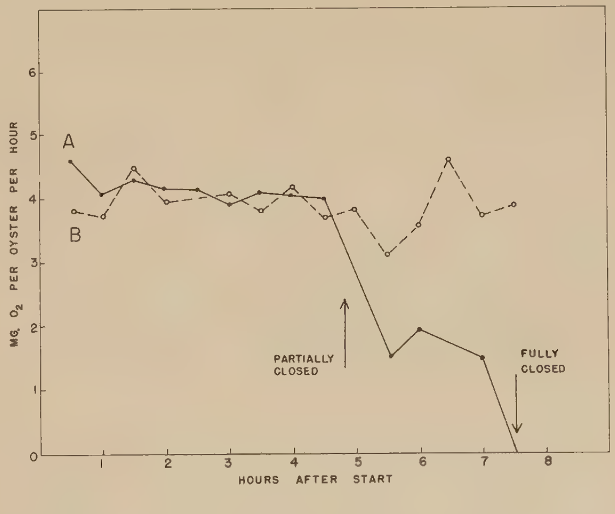

FIGURE 190.—Oxygen uptake, in mg. of oxygen per oyster per hour, measured at half hour intervals. Temperature 24.5° C.; salinity of water 31.8°/₀₀. Oyster A (solid line): wet weight of tissues 15.2 g.; dry weight 2.1 g. Oyster B (broken line): wet weight of tissues 12.8 g.; dry weight 1.5 g. Before the test these 6-year-old Long Island Sound oysters were kept for 30 days in Woods Hole Harbor. August.

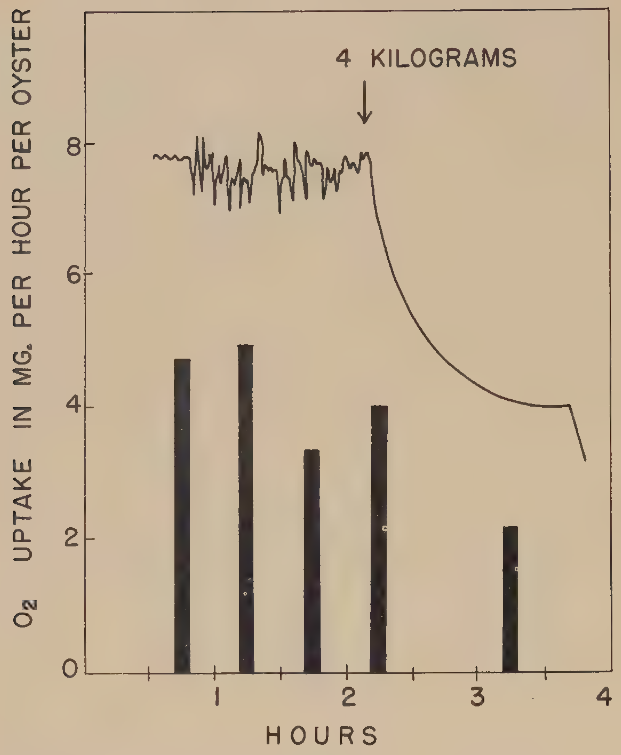

FIGURE 191.—Shell movements (upper line) and oxygen uptake (vertical bars) of an adult *C. virginica* before and during the application of a pulling force of 4 kg. Sharp inflection of the curve at about 3¾ hours corresponds to the point of tearing off the muscle. July experiment. Temperature 23.7° C.

their surface is not in direct contact with sea water. The high oxygen uptake after they are shed into the water is probably due to the greatly increased free surface areas of these cells.

In these observations the forcible stretching of the adductor muscle was always associated with a decrease and eventual cessation of ciliary current. Under the conditions of the tests the velocity of the cloacal current could not be measured, but decrease in current velocity was observed by the movements of particles suspended in the water or by the slanted position of the fecal ribbons, which in actively feeding oysters are horizontal to the axis of the cloacal current.

Two inferences can be deduced from these observations: First, the uptake of oxygen is dependent primarily upon the rate of water transport by the gills; and second, in maintaining a tonus level the locking mechanism of the adductor muscle is not dependent on the uptake of oxygen from surrounding water.

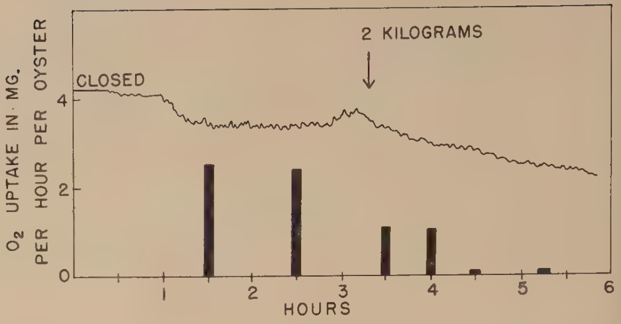

FIGURE 192.—Shell movements and oxygen uptake of an adult *C. virginica* before and after the application of a pulling force of 2 kg. Oxygen uptake in mg. per oyster per hour is shown by vertical columns. July. Temperature 22.4° C.

These observed rates of oxygen uptake are considerably greater than those found by Jørgensen (1952) for adult *C. virginica* of Woods Hole. The oysters used in his experiments were supplied from the Bureau's shellfish laboratory and were approximately of the same size as those which I tested in the preceding summers. The rate of oxygen uptake determined by Jørgensen was less than 1 ml. per hour (1.5 mg. of oxygen per oyster).

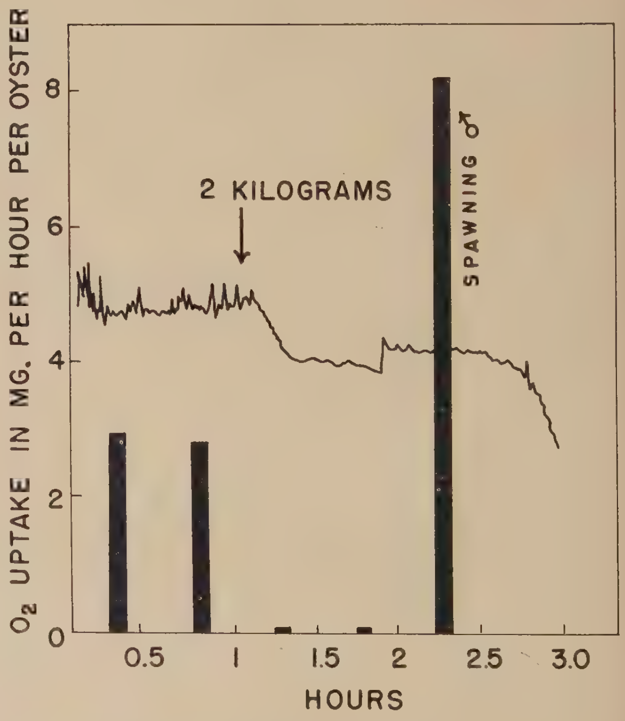

FIGURE 193.—Oxygen uptake of an adult oyster under normal conditions and after the application of a pulling force of 2 kg. Sudden increase of oxygen consumption at 2¼ hours is due to the discharged sperm. Upper line indicates shell movement. July. Temperature 24.8° C.

Unfortunately, Jørgensen does not mention the dimensions, weight, or conditions of his oysters, and does not describe the details of his technique. He states, however, (1952, p. 362) that "the oyster, *Ostrea virginica*, and the ascidians *Ciona intestinalis* and *Molgula manhattensis* filter about 10 to 20 l. of water for each ml. of oxygen consumed."

The low oxygen uptake reported by Jørgensen may have been due to the experimental conditions and particularly to the presence in the water of graphite particles used by him in determining the rate of water filtration.

Environmental effects

Seasonal changes in the rate of oxygen uptake.—After spawning the New England oysters pass through a period of lowered activity and tend to keep their valves closed, sometimes as long as 2 to 3 days; when they open again the rate of water transport is lower than it had been before the start of the reproductive period. Through the cold season of the year, from October to April, the oxygen uptake remains at a low level. In order to obtain comparable results and to eliminate the effect of temperature, tests of metabolic rates were made at 25° C., using oysters that were kept outdoors and brought into the laboratory for 3 to 4 days before testing to adjust gradually to the higher temperature. Examination of the data summarized in table 27 shows that the period of lowered metabolic activities occurred primarily during the winter.

Effect of change in salinity and pH.—No significant change in the respiratory rate was noticed in water of lowered salinity to which the oyster had become adjusted. In these tests the metabolic rate was first measured in water of 31.6 °/oo salinity. After the first test, which lasted 6 hours, the oyster was transferred for 3 days into running sea water diluted with fresh water to the salinity of 24.1 °/oo (approximately 76 percent of the previous concentration of salts). The rate of respiration was measured on the 4th day under standard conditions. The results of the test (figure 194) show that the rate of oxygen uptake in the water of lower salinity was not significantly different from that observed in 31.18 °/oo salinity. The tests were repeated several times with different oysters with identical results. In all experiments the oysters were left in water of lower salinity for at least 3 days to adjust to the new conditions. The effects of greater dilution of sea water have not been studied because of the technical difficulty in providing sufficient food to the oysters during the prolonged periods of adjustment.

TABLE 27.—*Seasonal changes in the oxygen uptake in mg. of oxygen per oyster per hour of adult Long Island oysters about 10 cm. long and 7 cm. wide*

Oyster No.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
50	5.0	4.2	----	----	----	2.6	----	2.6	2.3	----	----	2.0	2.7
51	3.8	----	----	----	----	----	----	2.9	----	----	----	2.1	----
52	3.5	1.5	----	1.8	----	----	1.9	2.4	1.6	----	----	1.7	----
53	4.4	----	----	----	----	----	----	----	3.6	----	----	----	----
55	5.2	----	----	3.0	----	----	3.6	----	4.0	----	2.7	4.7	4.3
56	----	4.1	----	----	2.4	3.1	----	----	2.9	----	----	4.4	2.9
62	4.7	----	----	2.6	----	----	2.0	----	3.1	----	----	5.4	4.3

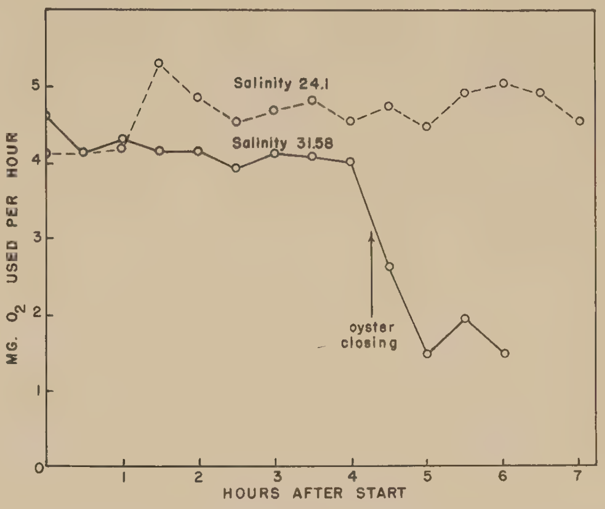

FIGURE 194.—Oxygen uptake of an adult Long Island oyster recorded at normal salinity (solid line) of Woods Hole (31.58 °/oo) and 4 days later at lowered salinity (broken line) of 24.1 °/oo. All tests were made in August under standard experimental conditions at the temperature of 25.0° C.

The pH has a very pronounced effect on the rate of oxygen uptake. The water used in metabolism tests was acidified by adding a quantity of 0.1 N hydrochloric acid. After six or eight readings with normal sea water, the acidified water was turned on and the testing continued for another 3 to 4 hours. The curve in figure 195 summarizes the results of all 10 tests performed in July to September using Long Island and Massachusetts oysters. At pH 6.5 the oxygen uptake drops to about 50 percent of the normal rate and rapidly decreases to less than 10 percent at pH 5.5. At pH 5.8 the oxygen uptake may continue for several hours at a greatly reduced rate (fig. 196).

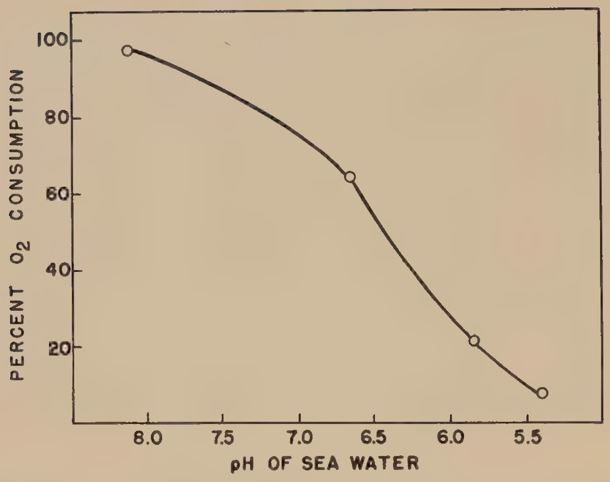

FIGURE 195.—Effect of lowered pH on the rate of oxygen uptake of adult oysters. Summary of summer experiments at Woods Hole at 25° C.

RESPIRATORY QUOTIENT, R.Q.

The respiratory quotient (R.Q.) is the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed at the same time. Definite values of R.Q. have been recognized for the principal types of food (Vernon, 1895; Richardson, 1929; Krogh, 1941). The R.Q. for carbohydrate is 1.00; for protein about 0.79; and for fat 0.71. On an average mixed diet the R.Q. of man is about 0.80 to 0.85. The herbivorous animals tend to have a higher R.Q., while the carnivorous have a lower one.

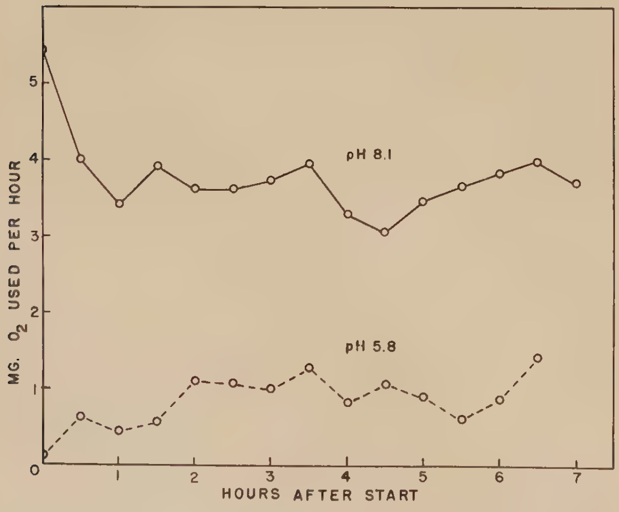

FIGURE 196.—Oxygen uptake of two oysters of approximately equal size (about 6 cm. in height) at pH 8.1 and 5.8. Salinity 31.6 °/₀₀. Measured for 6½ consecutive hours. Temperature 24.5° C.

It is of interest to find out whether the R.Q. of the oyster, which is an herbivorous mollusk, changes after the breeding season when it begins to accumulate and store glycogen. Orton (1927a, 1927b) suggested, without presenting supporting evidence, that during the reproductive cycle of *O. edulis* there is a shift from predominantly protein to carbohydrate metabolism and that this shift is correlated with the completion of the male sexual phase.

In conducting observations on carbon dioxide production it is necessary to keep in mind that deposits of calcium carbonate in the bodies of some marine invertebrates may suddenly release large quantities of this gas which would give a false R.Q. (Bosworth, O'Brien, and Amberson, 1936). For instance, the reported R.Q. 1.39 of lobster was found to be false since in lobsters coated with collodion the R.Q. was only 0.92. The shell of the bivalves is the principal storage place of carbonates which act as buffers when the valves are closed. In metabolism studies this possible source of error should be eliminated by coating the shells with paraffin.

The values of respiratory quotients vary during the conversion of food substances within the organism. Fattening of livestock and birds by forced carbohydrate feeding is usually accompanied by high R.Q., while the utilization of fats and proteins and their possible conversion to carbohydrates lowers the R.Q. values. It is, therefore, possible to expect that seasonal variations in the R.Q. of the oyster would give a clue to changes in the utilization of its food.

A Van Slyke constant volume apparatus for gas analysis was used in a series of observations of a group of marked oysters kept in live boxes in the harbor. No definite trend in the changes of R.Q. could be detected. It varied throughout the year from 0.51 to 1.44 as may be seen in the summary of observations (table 28) made under standard conditions in water containing no plankton. Addition of water soluble food in the form of dextrose resulted in an increase in R.Q. The latter determinations (table 29) were made in filtered water to which dextrose was added. There was no increase in R.Q. in water containing 0.0025 percent dextrose, but in 0.005 and 0.01 percent the R.Q. values were significantly higher.

RESPIRATION IN OTHER SPECIES OF OYSTERS

Comparison between the results described for *C. virginica* and the data published by others for

TABLE 28.—*R.Q. of* C. virginica *through the year*

[Long Island Sound oysters]

Oyster No.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
M-1		0.97	1.18									0.81
M-1		0.84										
M-1		0.97										
M-2							1.00					
M-2							0.83					
M-3							0.89					
M-4	0.92		0.97		1.06							
M-6	0.79				0.79		0.84					
M-6							0.69					
M-6											1.19	
M-7												0.57
M-9		0.51		0.57	0.82							0.50
M-9		0.71										
M-10	0.51			0.71								
R-50			0.72									
R-50			0.72									
R-51						0.55						
R-52	0.61		0.80			0.75				1.13		
R-52										0.97		
R-55	0.66		0.86		0.92	0.74					0.89	
R-56			1.35			0.84					0.64	
R-59			0.54									
R-59			0.44									
R-62	1.44		1.41			0.82					0.86	

other Ostreidae is difficult because of the different conditions under which the metabolism tests were made. In a discussion of the relation between the metabolism and temperature and its zoogeographical significance Spärck (1936) makes the statement that, "*O. edulis* consumes more oxygen than *Gryphaea* (*Crassostrea*) *angulata* of which it is shown that it is able to supplant *O. edulis* in several localities." This conclusion based on a few single determinations is not well substantiated.

Pedersen (1947) studied the respiration of *O. edulis* living in the small salt-water ponds along the Skagerrak coast in Norway. The summer temperature in these ponds rises to 25° C. and higher, while in winter ice covers the ponds for about 5 months. Prior to making the test Pedersen kept the oysters for a few days in filtered sea water, brushed them, washed the shells with 40 percent alcohol, and wrapped them in pieces of gauze to prevent small bits of shell from being broken off. For measuring the oxygen uptake the oysters were placed in hermetically closed glass containers filled with 2 l. of unfiltered sea water. The containers had to be turned over in order to mix the water. Closing and opening of shells were not recorded. Undoubtedly the turning of vessels caused the oysters to close their shells and discontinue the ventilation of the gills. For control Pedersen used blanks that contained no oysters. The difference between the blanks and the samples taken from the experimental containers was considered equal to the quantities of oxygen consumed and carbon dioxide produced by the oysters. The consumption of oxygen was expressed in mg. of oxygen per 100 g. of total weight or per 10 g. of net weight (presumably the wet weight of the meat) per 24 hours. Under these conditions and at temperatures around 24° to 25° C. the oxygen consumption of the oysters varied from 15.0 to 48.9 mg. of oxygen per 10 g. of weight per 24 hours or from 0.62 to 2.0 mg. per 1 hour. Pedersen's technique had serious drawbacks since the time the oysters were open is not known; the mollusks were disturbed by violent motions (turning over of the containers); and the animals probably were affected by accumulation of metabolites. The reported R.Q. of the Norwegian oysters varied from 0.8 to 1.0, but in some instances it was as low as 0.6 or as high as 2.6 and 3.0. The abnormally high and low values are probably fictitious because of some deficiency in technique.

TABLE 29.—*Effect of dextrose in sea water on R.Q. of* C. virginica

Date	R.Q. values in—		Percentages of dextrose
	Filtered sea water	Filtered sea water + dextrose	
Nov. 14	0.87	0.85	0.0025
Dec. 2	.57	1.56	.005
Dec. 10	.82	1.56	.01
Jan. 15	.92	1.38	.01

In a study of the energy-metabolism of *O. edulis* Gaarder and Eliassen (1955) used a closed chamber system (desiccators) of 750-ml. capacity. The shells of the oysters were kept open by glass rods inserted between the valves. The salinity of water was 32 °/₀₀, and the temperature was kept constant within ±0.05° C. The results were expressed in ml. of oxygen consumed per 1 g. of wet weight per hour. To facilitate the comparison I have recomputed the data of the Norwegian investigators to mg. of oxygen per 10 g. of wet weight of oyster tissues. At 25° C. the oxygen consumption by *O. edulis* computed on this basis was about 2.0 to 2.5 mg. of oxygen per hour per oyster.

As one may expect from observations on the effect of temperature on ciliary motion of the gill epithelium of the oyster, the oxygen consumption increases about 1.5 times for every 10° rise (Q_{10}) of temperature between 10° and 25° C. The maximum is reached at about 25° C. Below 5° C. the oxygen uptake decreases rapidly but measurable values were recorded by Gaarder and Eliassen even at temperatures approaching 0° C. It may be assumed that under normal conditions the valves would be closed at this low temperature and ventilation of the gill stopped.

Another experiment by Gaarder dealt with the effect of oxygen tension on oxygen consumption. The "critical oxygen tension" at which a decrease in the oyster oxygen consumption becomes apparent was found to be 4 ml. of oxygen per 1. (at 22° C.). If the figure is correct, it would indicate that *O. edulis* has a higher "critical point" than the one reported for *C. virginica* in which the rate of oxygen consumption begins to diminish when the oxygen tension is reduced to 2.5 cm.^{3} per 1. or lower (Galtsoff and Whipple, 1931). Gaarder and Eliassen disagree with Pedersen's (1947) conclusion that *O. edulis* can live for quite a while in water poor in oxygen. They think this species shows a rather high "critical point" of oxygen tension. In both *O. edulis* and *C. virginica* the uptake of oxygen is independent of oxygen tension above the respective critical points.

O. circumpicta, observed in a closed chamber system of about 8-l. capacity containing a thick layer of liquid paraffin, was found by Nozawa (1929) to consume oxygen at the rate of about 3.2 ml. of oxygen per hour per 10 g. of wet tissues. This rate is computed from Nozawa's published data with an assumption that his figures of oxygen uptake represent the cm.^{3} of oxygen. The R.Q. values of this species gradually increased during the 22 hours of observations from 0.85 to 2.8. The validity of the latter figure is questionable and is probably due to accumulation of metabolites. Nozawa claims that oxygen consumption of *O. circumpicta* is independent of oxygen tension until the latter is reduced to 0.1 percent of its normal content in water. The figure appears to be too low to be accepted without further verification.

UTILIZATION OF OXYGEN

Bivalves use only a small portion of the oxygen dissolved in the water which they transport through the gills. The percentage of oxygen consumed is the measure of the intensity of utilization of oxygen. In most cases less than 10 percent of the oxygen available is removed from the water (Hazelhoff, 1938). In comparison to gastropods and cephalopods, which utilize up to 80 percent of the available oxygen, the oxygen demand by bivalves is very low. The actual figures of utilizaton vary depending on the conditions of the mollusks. In *Mya arenaria* and in fresh-water *Anodonta* the normal utilization ranges from 2 to 10 percent (Van Dam, 1938). The low rate of utilization is due to the rapid transport of water which both mollusks have to maintain in order to obtain a sufficient supply of food. Van Dam reports (1937, 1938) that in many cases when the respiratory current was slowed down or when before making the test the mollusks were left in the air for 20 hours, as much as 97 percent of the oxygen was utilized.

The rate of oxygen consumption is usually higher after a period of interruption of respiratory current or after exposure of mollusks to air. This compensation by oysters for an oxygen debt has also been observed in *Mya arenaria*, and in *Anodonta cygnea* (Koch and Hers, 1943). The authors maintain that the rate of ventilation of the gills of *Anodonta* is regulated both by the need of the animal for oxygen and by the availability of oxygen in water. Oxygen determination in their experiments was made by means of a polarograph. By this method is was possible to record photographically the continuous changes in the oxygen content of water of the exhalant current. The inference the authors draw from their observations is that the regulation of the branchial current in *Anodonta* by contraction of the exhalant siphon has relation to the intensity of metabolic processes. They found that the periods of closure of siphons are longer in water rich in oxygen and become shorter when the oxygen content is low. The technique of dropping mercury electrodes (Petering and Daniels, 1938; Føyn, 1955; Brezina and Zuman, 1958) appears to be promising and should be applied in further study of respiration in mollusks.

The coefficient of oxygen utilization in the oyster (percent of oxygen removed from the water as it passes through the gills) has not been determined. The data of the metabolism tests cannot be used for this purpose because the actual rate of water transport cannot be measured in an oyster kept in the respiratory chamber. The flow of water through the chamber was maintained at a rate lower than the expected rate of water transport through the gills and, consequently, it is reasonable to expect that the water in the chamber passed through the gills several times before it reached the outlet.

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CHAPTER X

ORGANS OF DIGESTION AND FOOD OF THE OYSTER

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The system of organs concerned with the ingestion and digestion of food and elimination of feces consists of the mouth, short esophagus, stomach, crystalline style sac, digestive diverticula, midgut, rectum, and anus (fig. 197). With the exception of a short section of the rectum, the entire alimentary canal lies within the visceral mass and is completely immobilized by the surrounding connective tissue. In the absence of peristaltic movements the food is moved from the mouth toward the anus exclusively by the strong ciliary motion of the epithelial lining of the system. It is difficult to reveal all anatomical details of the alimentary tract by dissecting the tissues. Better preparations can be obtained by making casts of latex or other suitable material injected through the esophagus or via the rectum and left until it sets. Yonge (1926a) used for this purpose a warm, concentrated solution of gelatin. Awati and Rai (1931) employed a mixture of paraffin and resin colored with carmine. Satisfactory results were obtained in the Bureau's shellfish laboratory by using red or yellow latex injected into the esophagus through a wide mouth pipette with a rubber balloon. The preparation was immediately placed in 5 percent formalin in which the latex sets and remains tough and flexible for a long time. Casts of the entire alimentary canal or of its parts can be obtained in this way.

MOUTH

The mouth is a compressed U-shaped slit between the two lips (fig. 104) and is lined with columnar ciliated epithelium set on a narrow basal membrane. The epithelial cells of the mouth are taller than those of the labial palps and contain only a few mucous glands. In the surrounding connective tissue are large vesicular cells, numerous muscle fibers, and blood spaces which are occasionally filled with leucocytes. Leucocytes are also found in narrow spaces between the tissue cells and on the surface of the mantle lining from which they are discarded.

ESOPHAGUS AND STOMACH

The esophagus, a short, funnel-shaped, and dorso-ventrally compressed tube, is lined with epithelium similar to that of the mouth. It leads to the stomach, which occupies a central position within the visceral mass (fig. 197). The stomach is an irregularly shaped, large sac (figs. 198 and 199) with several outgrowths or pouches. At the entrance of the esophagus the wall of the stomach forms an anterior chamber, a, which leads into a broader posterior chamber, b (figs. 198 and 199). An oblique outgrowth or pouch called the caecum, c, is the most conspicuous structure which arises from the ventral side of the anterior chamber. Both the anterior and posterior ends of the caecum are curved and form the anterior and posterior appendices (a.ap., p.ap.). The larger posterior appendix is a strip curved ventrally and toward the right of the stomach. The configuration and relative sizes of the appendices vary but the structures are recognizable in all the casts. A groove along the wall of the caecum leads to the opening of the midgut (m.g.) and serves for sorting of food (Yonge, 1926a). On the left side below the caecum the wall of the stomach bulges out to form a broad pyloric caecum (p.c.), which leads to a long outgrowth alongside the midgut, the crystalline sac (cr.s.).

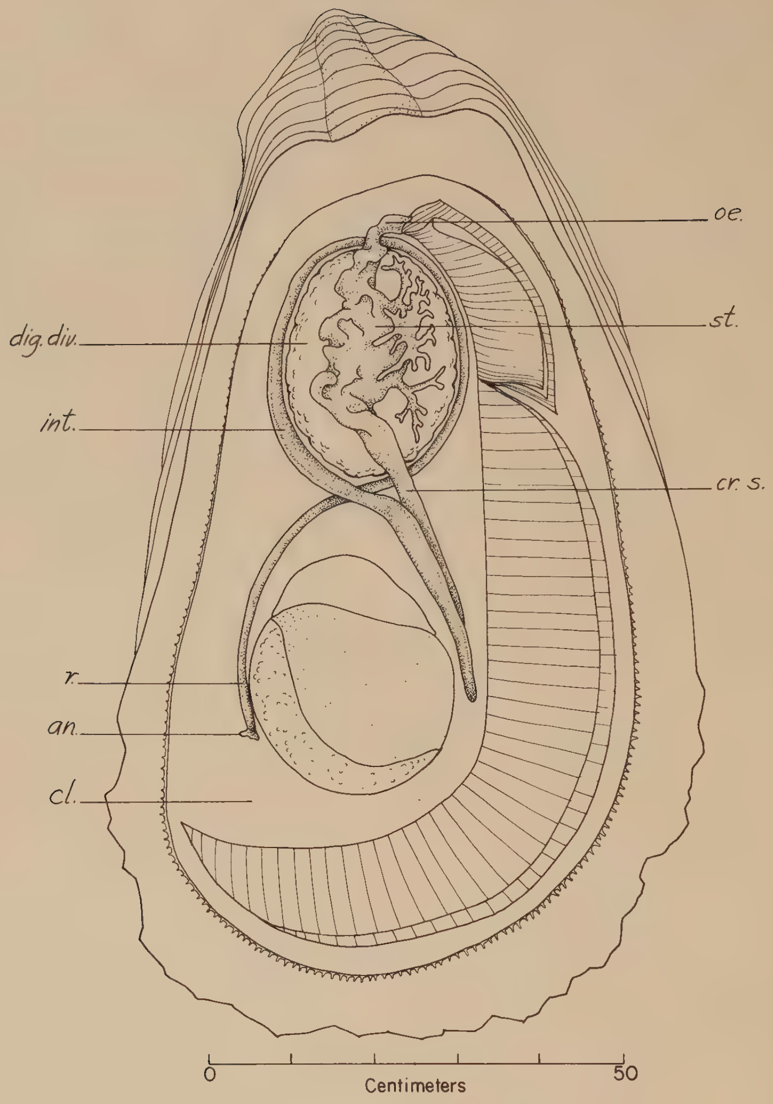

FIGURE 197.—Digestive system of the oyster, *C. virginica*, drawn from the dissected preparation after the injection of latex. The right outer labial palp was cut off to expose the esophagus. The parenchymal tissues over the stomach and intestine were removed. an.—anus; cl.—cloaca; cr.s.—crystalline style sac; dig. div.—digestive diverticula; int.—intestine (mid-gut); oe.—esophagus; r.—rectum; st.—stomach.

Three groups of wide ducts emerging from the wall of the stomach lead to the digestive diverticula. Two of them (fig. 199. d_1, d_2) originate at the anterior chamber and one (d_3) from the posterior chamber.

The internal lining of the anterior chamber forms a number of irregular ridges and furrows covered with ciliated epithelium. A broad ridge separates the anterior from the posterior chamber and apparently directs the food particles. The left ventral wall of the posterior chamber is covered by a translucent membrane, the gastric shield (fig. 200), which lies directly opposite the opening of the long sac occupied by the crystalline style (cr.s.).

Ciliary tracts of the stomach lining are very complex. Detailed observations on the course followed by food particles after they enter the stomach were made for *O. edulis* and *Mya arenaria* by Yonge (1923, 1926a), who studied them by carefully cutting off the wall and adding fine powdered carborundum or aquedag to the exposed surface. In general the pattern of ciliary movements in the stomach of the American oyster is similar to that of *O. edulis*. The direction of ciliary beat along different ridges and channels

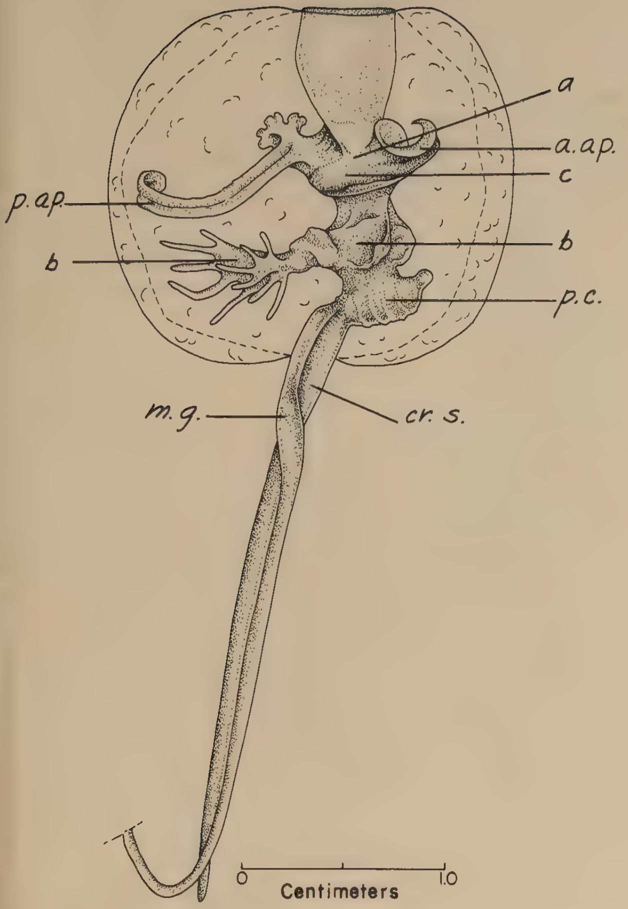

FIGURE 198.—Latex cast of the stomach, crystalline style sac, and esophagus of a large *C. virginica,* viewed from the left side. Upper part of the esophagus is slightly distended by injection. The visceral mass was dissected and the injected parts left in their natural position. Drawn from a preparation preserved in 5 percent formalin. a.—anterior chamber; a.ap.—anterior appendix of the caecum; b.—posterior chamber; c.—caecum; cr.s.—crystalline style sac; d.p.—dorsal pouch; d.—group of ducts of the digestive gland; m.g.—midgut; p.ap.—posterior appendix of the caecum; p.c.—pyloric caecum.

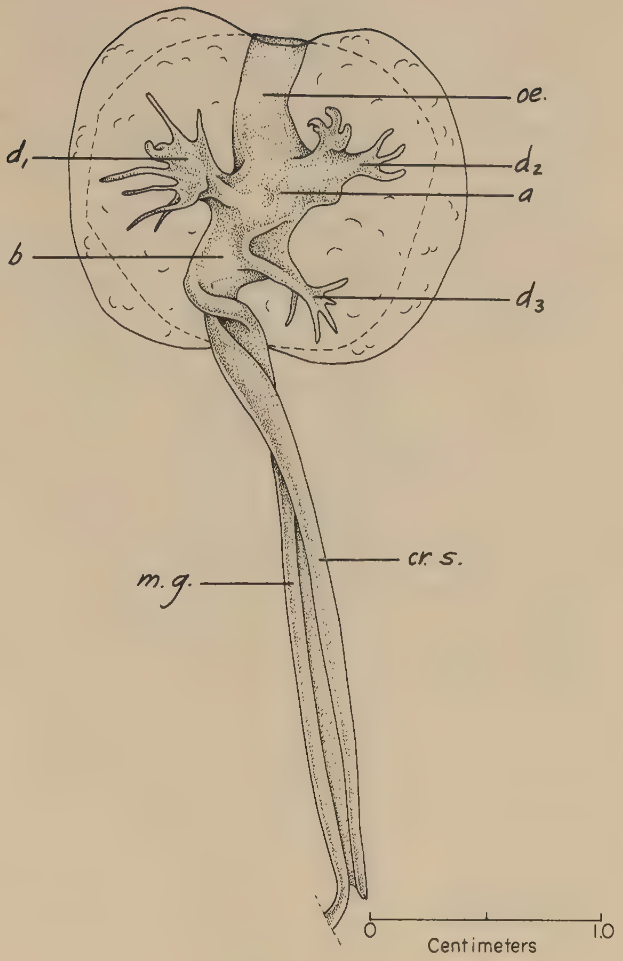

FIGURE 199.—Latex cast of the stomach, crystalline style sac, and esophagus of a large *C. virginica* viewed from the right side. The visceral mass was dissected, and the injected parts were left in their natural position. Drawn from a preparation preserved in 5 percent formalin. a.—anterior chamber; b.—posterior chamber; cr.s.—crystalline style sac; d_1, d_2, d_3—ducts leading to the digestive diverticula; m.g.—midgut; oe.—esophagus.

brings the food from the esophagus to the caecum where the food materials are separated according to size. Some of the larger particles entering the midgut may be voided without being digested while the smaller particles are pushed toward the gastric shield. Other groups of cilia conduct the particles toward the ducts leading to the digestive diverticula. The ducts branch out into a large number of smaller passages that ramify and extend deep into the mass of diverticula.

Nearly the entire inner surface of the stomach is covered by ciliated epithelium; only the areas under the gastric shield and near the posterior end of the stomach are nonciliated. The epithelium is of columnar type with very long cilia, which are particularly prominent on the ridges (fig. 201). The height of the cells gradually decreases toward the caecum. Mucous cells are abundant, particularly near the junction with the midgut, and phagocytes are numerous between the epithelial cells and in the underlying connective tissue. There is no well-developed muscular layer under the epithelial lining, but a few smooth

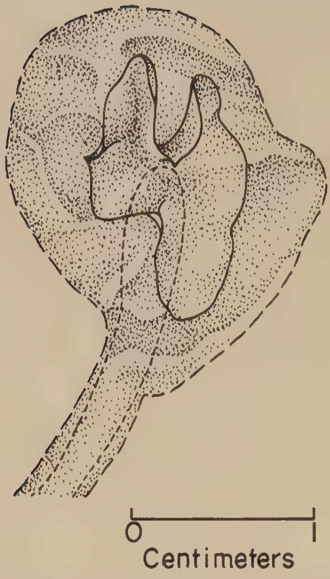

FIGURE 200.—Gastric shield viewed in its natural position in a dissected stomach. The position of the crystalline style is indicated by the dotted line. Drawn from an unpreserved preparation.

muscle fibers may be found under the basement membrane. In general, the histological picture of the stomach of an adult *C. virginica* is similar to that described for the spat of this species by Shaw and Battle (1957), *C. angulata* by Leenhardt (1926), and *O. chilensis* and *O. edulis* by Dahmen (1923) and Yonge (1926a) respectively.

GASTRIC SHIELD

The stomach wall in front of the openings to the midgut and style sac is covered by a thin but tough, irregularly shaped membrane (fig. 200) made of translucent and slightly striated material. The structure, named the gastric shield by Nelson (1918), rests on a prominent epithelial ridge of narrow columnar cells with oval nuclei, rich in chromatin (fig. 202). The cells are devoid of cilia. The shield is made of two portions of different size, joined together by a narrow middle piece (fig. 200). The thicker portion of the shield lies over the peak of the ridge and is underlined by the tallest cells in the area. On both sides of the peak the epithelium flattens and at the edges changes into the typical ciliated lining of the stomach. The surface of the shield is roughened by the remnants of food particles embedded in it.

The origin of the shield has not been fully explained. Obviously, it is the product of the underlying cells, but the process of its formation has not been studied sufficiently. One view, advanced by Gutheil (1912) and shared by some investigators, assumes that the shield is formed by the droplets secreted by the epithelial cells. No evidence in support of this view can be found in the histological preparations of the stomachs of *O. edulis* and *C. virginica*. No droplets could be seen in the sections of stomach, and no other indication of the secretory activities of these cells could be found. Yonge (1926a) thinks that the shield is very likely formed by the fusion of the cilia and in support of this view points out that the structure is attached to the epithelium by fine threads which transverse the substance of the shield and resemble the cilia. Indistinct transverse striation can be seen in the sections of the stomachs of *C. virginica* fixed in osmic acid and stained with iron hematoxylin (fig. 202). The question could be settled by electron microscopy, which would reveal the structure of the cilia if the latter are present within the shield substance. So far no such studies have been made.

The shield is not destroyed by boiling in a 40 percent solution of potassium hydroxide. Treatment with iodine followed by a strong solution of zinc oxide gives the deep violet coloration that is characteristic of the color reaction for chitin (Zander reaction). These facts support Berkeley's (1935) findings that the material of the shield of the common Pacific coast clam, the Pacific gaper (*Schizothaerus nutalli nutalli* Conrad), is made of chitin and contains no chondrinlike constituent.

In *C. angulata* Leenhardt (1926) described the torch bearing cells near the edges of the area occupied by the gastric shield. The function of the cells is not known. They are not found in my preparations of *C. virginica* and are not mentioned

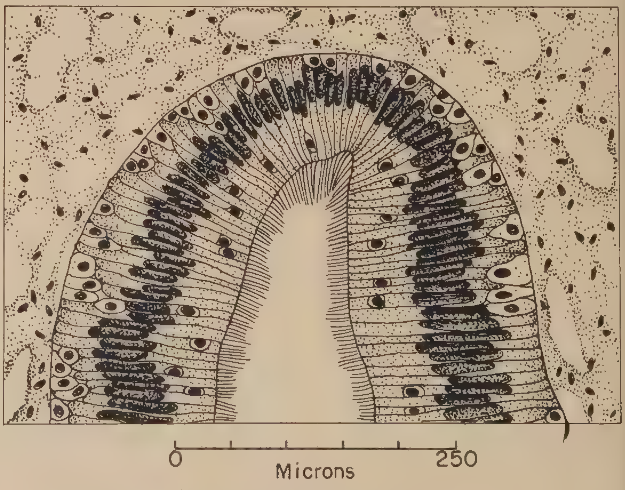

FIGURE 201.—Cross section of the wall of the stomach. Kahle, Hematoxylin-eosin stain.

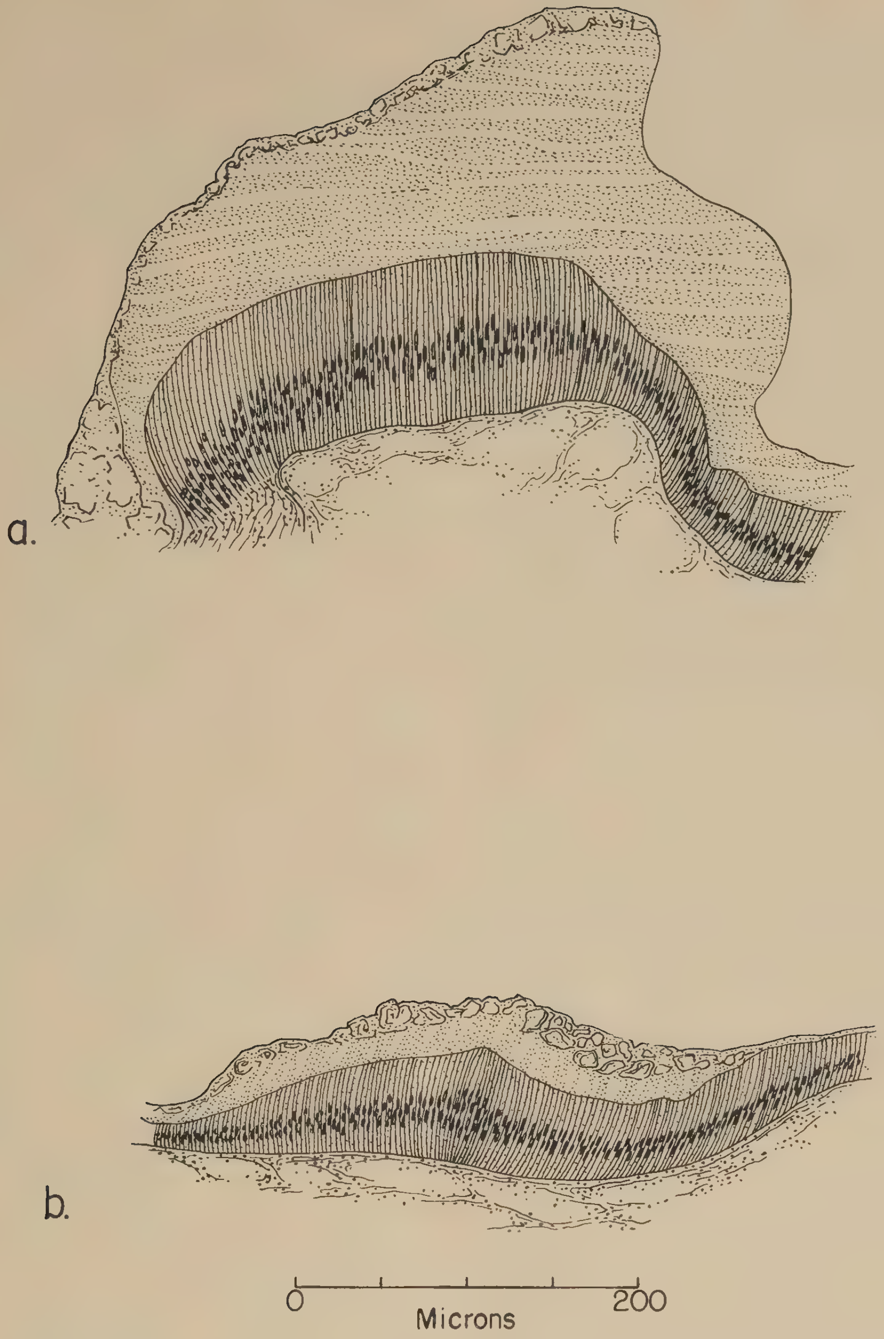

FIGURE 202.—Two cross sections of the wall of the stomach of *C. virginica* under the gastric shield. A—the thickest portion of the shield. Bouin, hematoxylin-eosin stain. B—cross section near the periphery of the shield. Osmic acid, iron hematoxylin. The surface of the shield is rough due to embedded and partially ground food particles. Note cross striation of the shield visible in B.

by Shaw and Battle (1957) in their work on the microscopic anatomy of the digestive tract of this species.

The function of the shield is to provide a base for grinding of food by the rotating head of the crystalline style.

CRYSTALLINE STYLE

The posterior wall of the stomach leads to an elongated outgrowth or sac which extends a considerable distance along the ventral arm of the visceral mass (fig. 197, cr.s., and fig. 203) on the antero-ventral side of the adductor muscle. A narrow slit joins the sac over nearly its entire length to the midgut; near the entrance to the stomach the two structures are separated. The sac is slightly twisted around the midgut and occupies a somewhat dorsal position, while the midgut forms the ventral portion of the common structure (figs. 198, 199). A cross section of the sac and midgut shows (fig. 204) that the two channels are separated in the middle by a narrow slit compressed by the two protruding lobes or typhlosoles. In figure 204 the style sac is at the top; its lumen is usually larger than that of the midgut (lower part of the figure). This relationship between the style sac and midgut is similar to the topography of this organ in *O. chilensis* (Dahmen, 1923), *O. edulis* (Yonge, 1926a), *Mya* (Edmondson, 1920), *Ensis* (Graham, 1931a), *Mytilus edulis* (Sabatier, 1877), *M. latus* and *M. magellanicus* (Purdie, 1887), and *Anodonta* (Nelson, 1918). In the old literature the structure was called a "tubular stomach" by Sabatier (1877), and "pyloric appendix" by Purdie (1887), names which have not been accepted in malacological literature.

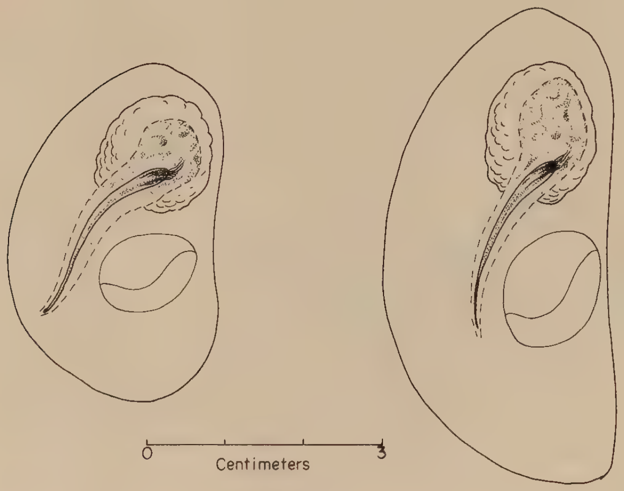

Figure 203.—Crystalline styles of *C. virginica* (left) and *C. gigas* (right) in their natural position. Drawn from live preparations. The wall of the stomach and of the crystalline style sac has been dissected.

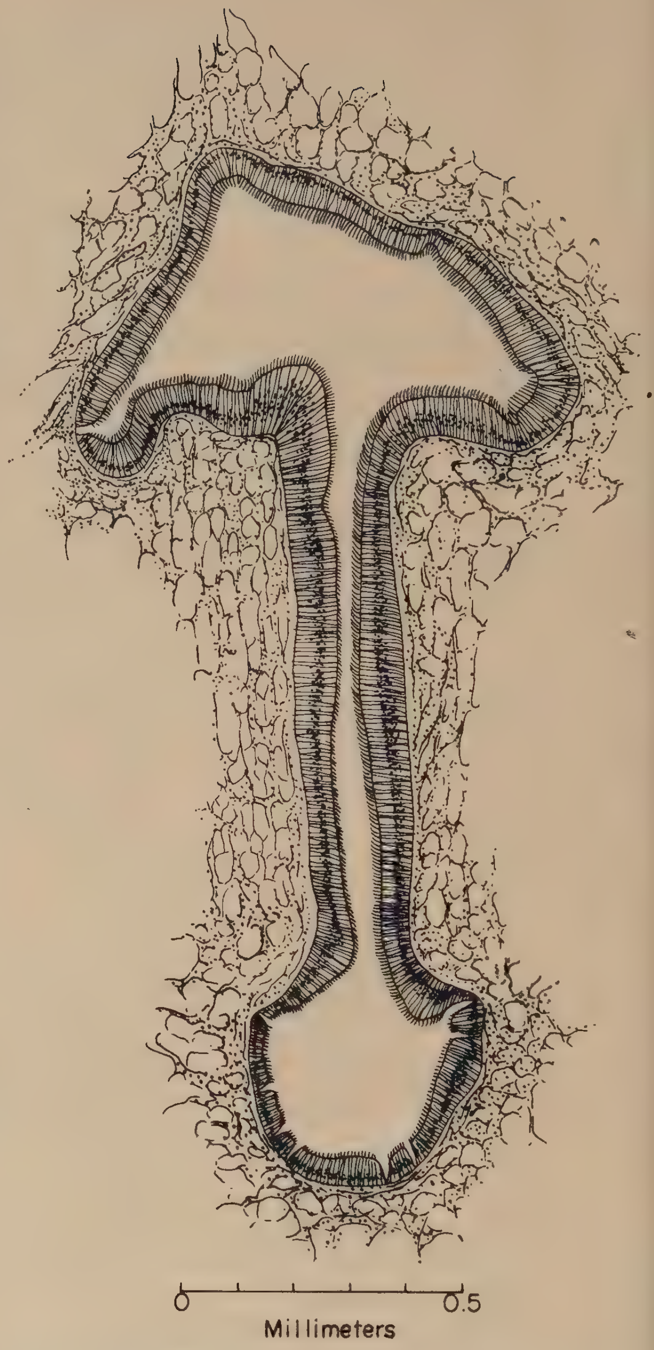

Figure 204.—Transverse section of the style sac (upper part) and midgut (lower part). The crystalline style is absent. Kahle, hematoxylin-eosin.

The style sac is lined with densely packed cylindrical cells that have large oval nuclei and long cilia measuring about 20 μ. The intracellular fibrillar apparatus is well developed. Phagocytes and mucous cells are scarce. The basal membrane rests on a thin layer of collagenous fibers; circular muscles are sparsely arranged, as in the stomach, and there is no distinct muscular layer. The epithelial cells of the two lobes (typhlosoles) of the sac and midgut gradually change from robust, long cells to shorter cells with smaller cilia, typical for the lining of the midgut. The mucous cells are more abundant in the midgut than in the sac.

The connective tissue around the sac and under the typhlosoles consists of typical vesicular cells.

In actively feeding oysters the lumen of the sac is occupied by a gelatinous rod with a bulging head protruding inside the stomach (fig. 203) and the pointed tail extending to the distal part of the sac. The color of the style varies from greyish white to deep yellow and brown, depending on the type of food consumed by the oyster. The head is usually darker than the rest of the style because of the food particles wrapped around it.

Inside the sac the style is rotated by the ciliary action of the epithelium. The rotary motion was originally suggested by List (1902) in his work on mussels, but the demonstration that the rotation actually takes place in *Anodonta* and *Modiolus* was made by Nelson (1918). According to Yonge (1926a), the large cilia of the groove of the sac of *O. edulis* move in such a way as to produce a slow clockwise rotation of the style when seen from the stomach. There is, however, a tract of cilia on the side of the larger typholsole which beats in the direction of the stomach and presumably pushes the style forward. Food particles that enter the sac are carried by the currents down the gut but some of them tangle in the substance of the style, are wrapped around it, and carried back to the stomach. This process, observed by Nelson (1918, 1925), Allen (1921), and Orton (1924), may be significant for the bivalves in which, like in *Ostrea*, the style sac is in direct communication with the midgut.

As the style rotates and rubs against the gastric shield, aiding in mixing and grinding food particles it slowly dissolves in the gastric juice and yields digestive enzymes.

FORMATION

The crystalline style is not a permanent structure. In oysters removed from water and left in the air the style dissolves in a short time. This observation, reported for *O. edulis* by Orton (1924), has been confirmed for *C. virginica* and *C. gigas*. At room temperature of 21° to 22° C. the crystalline styles of the American species removed from the sac and left exposed to air completely dissolve within 45 to 60 minutes. In the body of the oysters (*C. virginica*) taken out of water the style disappears in 2 to 3 hours. The absence of the style is frequently observed in nonfeeding oysters. The symptom is useful, but not entirely reliable because under certain conditions the style may be present in oysters which do not take food. Observations made in winter in the Woods Hole laboratory showed that in late December, at temperatures varying from 5.4° to 5.7° C. about 4 out of 10 oysters had crystalline styles. No trace of food was found in these oysters, which were examined within a few minutes after they had been taken out of water.

Yonge (1926a) states that in *O. edulis* the style is always present in healthy oysters, even when they are starved, and is absent only under abnormal conditions and in diseased specimens.

The style must be the product of secretion but investigators do not agree on the manner and site of its formation. List (1902), Nelson (1918), Edmondson (1920), and Mackintosh (1925) think that the style is secreted by the narrow cells of the smaller typhlosoles but do not present conclusive evidence in support of this view. For freshwater *Anodonta*, Gutheil (1912) demonstrated the presence of vesicular granules around the nuclei of the epithelial cells of the sac and probably interpreted them correctly as a sign of active secretion. No such granules were found, however, in the histological preparations of *O. edulis* (Yonge, 1926a) and in my slides of the sac of *C. virginica*. Evidence of the secretory activity of the style sac was produced by Yonge (1926a) by injecting 0.5 percent solution of iron saccharate into the adductor muscle, washing the animals, and then dissecting and fixing the sac at 2-hour intervals. The sections were treated by potassium ferricyanide in acid solution to demonstrate the presence of iron by Prussian blue reaction. Fine blue granules indicative of the presence of iron salt were found in the cytoplasm above the nuclei and between the cilia of the epithelial cells. No iron was detected in the epithelium of the midgut or of the larger typhlosoles, although some of the metal was present in the epithelial cells of the minor typhlosole. The experiments may indicate the secretory function of the epithelial cells, but they cannot be considered as evidence of the formation of the style from the secreted granules.

CHEMICAL COMPOSITION

Analysis of the crystalline style of *Cardium* made by Barrois (1889, 1890) showed the following composition: water 87.11 percent; solid organic matter 12.03 percent; solid inorganic matter 0.86 percent. The organic component of the style was considered to be a globulin with traces of mucus or chondrinlike substance. Berkeley (1935) demonstrated that the styles of four species of bivalves (*Crassostrea gigas, Mya arenaria, Schizothaerus nuttalii*, and *Saxidomus giganteus*) in addition to protein, yield, on acid hydrolysis, glucionic and sulphuric acids and a hexamine, the essential constituents of both mucin and chondrin. The ease of the hydrolysis and the solubility of the style materials indicate that mucin rather than chondrin is involved. The variations in the solubility and the quantitative differences in the chemical composition of the styles suggest, according to Berkeley, that the less readily soluble styles contain larger quantities of mucin.

All examined styles are carriers of certain enzymes which they yield upon dissolution. The role the styles play in digestion is discussed later (see p. 230 of this chapter).

MIDGUT AND RECTUM

The portion of the intestine between the stomach and the rectum is called the midgut. It begins at the ventral wall of the stomach next to the opening of the crystalline sac and runs parallel to the sac as far as its distal end, then turns sharply backward parallel to its previous course (fig. 197, in t.). The ascending branch of the intestine makes a loop that completely encircles the stomach and continues as a descending branch which ends with the rectum and anus (r., a.).

Throughout its entire length the midgut is characterized by a well-developed typhlosole which extends along its inner wall (fig. 205). The gut is lined with columnar ciliated epithelium; there is an abundance of mucous cells and of wandering leucocytes. The muscular layer is absent.

The rectum (fig. 197, r.) runs along the dorsal side of the heart. In this respect the oyster differs from many other bivalves (sea mussels, clams, fresh-water mussels) in which the rectum runs through the heart. The structure of the rectum is similar to that of the midgut; the main difference is the disappearance of the well-developed typhlosole near the anal region where the lining is thrown into numerous small folds (fig. 206). A distinct feature of the rectum is a circular layer of smooth muscles which, however, do not form a sphincter at the anus (fig. 207). According to Leenhardt (1926), the anal sphincter is present in the Portuguese oyster. The surface epithelium of the anal region is well developed and abounds in mucous cells.

DIGESTIVE DIVERTICULA

The stomach is surrounded by an irregularly shaped mass of dark tissue which has been called the "liver" or "hepatopancreas." Its color varies from light yellow to dirty green and dark brown. In most cases the color is not visible through a white or creamy layer of connective tissues rich in glycogen.

Yonge (1926b) has shown that assimilation and intracellular digestion takes place in this mass of darkly colored tissues; it has none of the known functions of the liver or pancreas. He named it "digestive diverticula", a term that correctly describes its role.

The digestive diverticula are made of a large number of blind tubules emptying into several large ducts which lead to the interior of the stomach. The structure of the tubules is simple. In cross section (fig. 208) they are usually round with a lumen in the form of a cross. The tubules are surrounded by connective tissue in which muscle fibers are absent. The digestive cells which form the interior of a tubule are large and well vacuolated, with large nuclei at their base. Food vacuoles can be seen in them during feeding. At the corners of the "cross" of the lumen one usually finds crypts of young cells with dark staining protoplasm, large and compact nuclei, and indistinct cell boundaries. Cells from these crypts replace the old cells that are cast off. The digestive cells of the American oyster are nonciliated, but the cells in the diverticula of other bivalves (*Teredo*) have been reported to have cilia (Potts, 1925). Yonge (1926a, 1926b) believes the cilia are present in the tubules of *edulis* but probably retract so rapidly that they cannot be seen in the fragments of tissues pressed by a cover slip. I was not able to detect them in *C. virginica*. Phagocytes are very abundant between the cells and in the surrounding connective tissue.

The ducts that connect the tubules with the stomach are circular in cross section and are lined with ciliated epithelium (fig. 209). Their lumen is, however, irregular due to the variations in the height of the epithelial cells. The epithelium is similar to that of the stomach and contains many mucous glands and phagocytes.

ALIMENTARY TRACT AND FORMATION OF FECES

Food ingested by the oyster is moved through the alimentary canal by the ciliary action of the

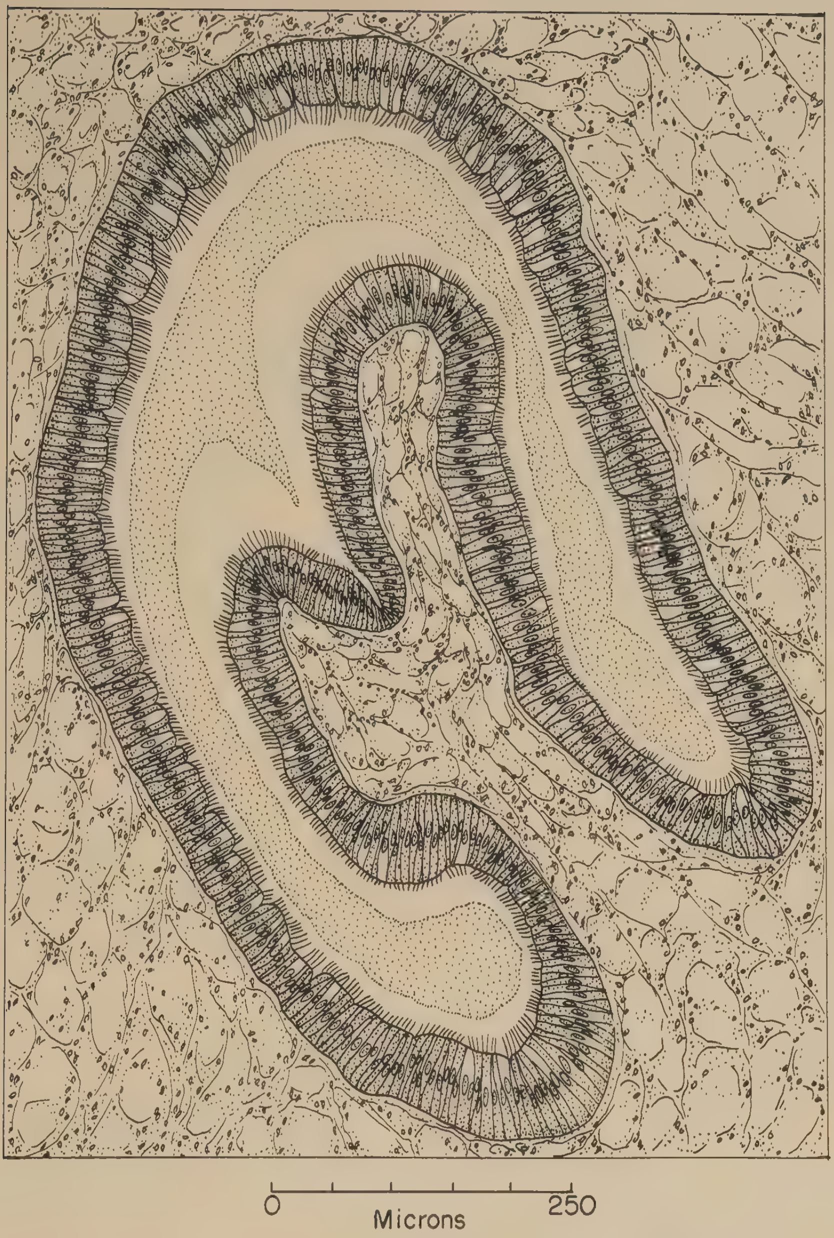

FIGURE 205.—Cross section of the midgut. Bouin, hematoxylin-eosin.

epithelium. There is no peristaltic motion since the muscular layer of the intestines is either absent or poorly developed, and the feces are discharged in a continuous ribbon which is carried away by the cloacal current and eventually settles. The time required for food to pass through the entire intestinal tract can be measured by recording the time between addition of a suspension of carmine or yeast to the gills and the appearance of the red or white particles in the feces. The rate of passage naturally depends on the length of the intestinal tract and the rate of feeding. In large oysters (about 10 by 6 cm.) kept in running sea water of about 15° to 16° C. the time required for food to pass through the entire intestinal tract varied from 90 to 150 minutes. The length of the intestinal tracts of the oysters used in these tests was measured on latex casts which were left in situ and exposed by dissecting the tissues above them. The lengths of the alimentary tracts were as follows:

In an oyster measuring 11 by 6 cm	14.5 cm.
In an oyster measuring 10.0 by 7.5 cm	11.1 cm.
In an oyster measuring 11 by 6 cm	12.9 cm.
In an oyster measuring 11.5 by 5.5 cm.	12.6 cm.

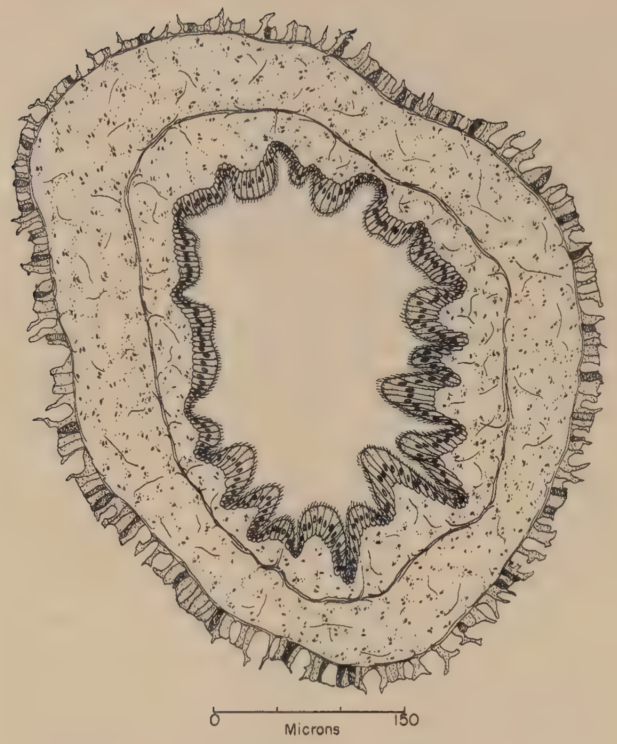

FIGURE 206.—Cross section of the rectum near the anal region. Bouin, hematoxylin-eosin.

The rates of discharge of fecal ribbons observed on actively feeding oysters in laboratory sea water of 15.0° to 15.7° C. are given in table 30. The average of the observed rate was 8.1 cm. per hour. Assuming that the average length of the intestines was 12.8 cm., the estimated time of passage of food through the entire alimentary tract was 95 minutes.

The feces of the oyster are voided from the rectum as a compact and slightly flattened ribbon of sufficient consistency to withstand the velocity of the cloacal current. In an actively feeding oyster the ribbon is maintained in a horizontal position along the axis of the current, but being heavier than the sea water it sinks down to the bottom as soon as the cloacal current slows down or ceases. Large masses of fecal ribbons accumulate on the bottom a short distance from the opening of the cloaca. The ribbon remains intact for 2 to 3 days until it is disintegrated through decomposition and mechanical disturbance. The appearance of the fecal masses of the oyster is typical and can be recognized by their shape. It was shown by Moore (1931) that specific identification of fecal pellets can be made for a number of marine invertebrates.

Fecal ribbons of oysters contain many live cells—diatoms, dinoflagellates, yeast, and others which are not killed by the gastric and intestinal juices and can be recultured.

TABLE 30.—*Rate of formation of fecal ribbons (in cm.) in* C. virginica *during feeding in laboratory sea water, Woods Hole*

Date	Time	Temperature	Length of ribbon	Rate of formation
	Minutes	*°C*	*Cm.*	*Cm./hr.*
May 18	108	15.6	23	7.7
18	17	15.0	3.2	11.3
18	102	15.0	9.8	5.8
May 19	70	15.2	14.2	12.2
19	123	15.0	9.3	4.5
May 23	60	15.7	7.2	7.2

DIGESTION

The digestion and absorption of food in the oyster are primarily an intracellular process which takes place in the digestive diverticula. This was demonstrated by Yonge (1926a, 1926b) in a series of carefully executed feeding experiments in which the solutions of iron saccharate, suspension of carmine powder, oil emulsion, and dogfish blood corpuscles were fed to European oysters. He produced convincing evidence that very small food particles are absorbed by the cells of the digestive diverticula, while the diatoms and other

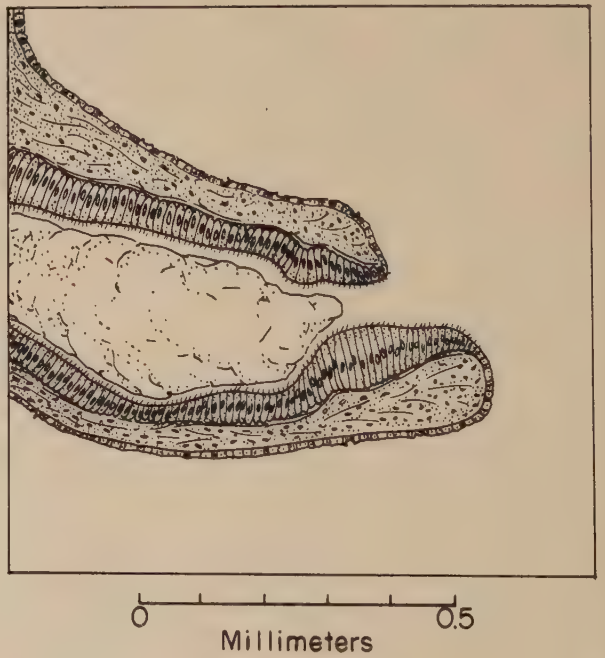

FIGURE 207.—Longitudinal section of the anus and adjacent portion of the rectum. Note the fecal mass inside and the absence of a sphincter. Bouin, hematoxylin-eosin.

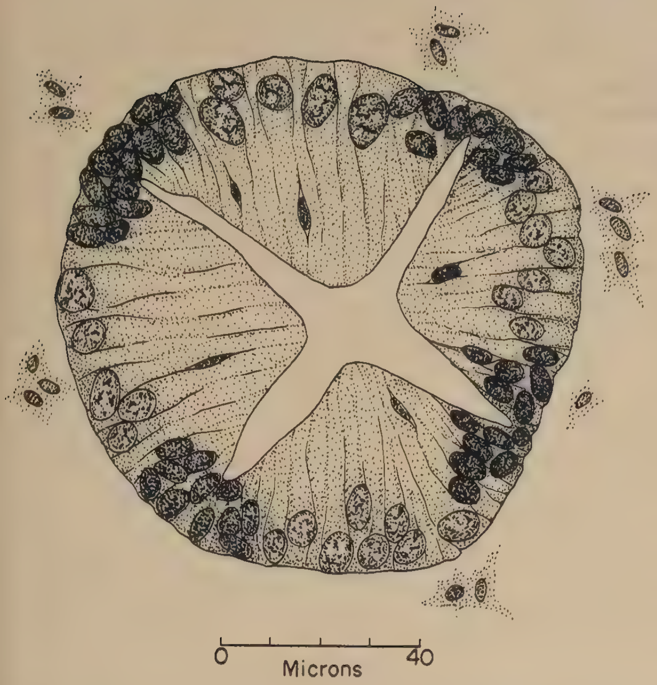

FIGURE 208.—Cross section of a single digestive diverticulum. Note the crypts of young cells at the corner of the cross of the lumen. Kahle, hematoxylin-eosin.

algae of larger size are ingested by phagocytes. Yonge's work fully confirmed the idea, first expressed by Saint-Hilaire (1893), that the digestive diverticula are the organs of absorption. He found no evidence of any secretion from the diverticula and demonstrated the importance of phagocytes in the digestive processes. Since the work of the earlier investigators is fully discussed by List (1902) and more recent investigations are summarized in several papers of Yonge (1926a, 1926b), the reader interested in the history of the problem is referred to these publications.

The digestion of food also takes place in the stomach where several digestive enzymes are present. On the basis of our knowledge, which admittedly is not complete, the process of digestion seems to take the following course. After being sorted several times by various mechanisms of the gills and labial palps, the food particles enter the stomach where the sorting continues and the larger particles are broken by the combined action of the crystalline style rotating against the gastric shield and the chemical action of enzymes which dissolve from the style. Very small particles are pushed by the cilia through the ducts into the digestive tubules where they are taken into the vacuoles of the digestive cells and are acted upon by the enzymes of these cells. Usable material is ingested by the phagocytes or is stored in the surrounding connective tissue. Indigestible substances like colloidal carbon of india ink are expelled. Some of the food particles, especially of larger size, are engulfed by the phagocytes which abound in the digestive tract. Circulation of food in the ducts is maintained by the ciliated cells.

The stomach contains free enzymes which are dissolved from the crystalline style. The most active among them are the amylase and glycogenase which digest starch and glycogen. Yonge's experiments (1926a, 1926b) showed that the optimum activity of oyster amylase is at approximately pH 5.9. Purification by dialysis or with absolute ethyl alcohol inactivates the enzyme, but its action can be restored by the addition of chlorides or bromides. Besides these two enzymes, the style contains a complete oxidase system. The presence of oxidases in the extract of styles was first demonstrated by Berkeley (1923) in the Pacific coast clam, *Saxidomus giganteus*, in rock cockle, *Paphia staminea*, and in soft-shell clam, *Mya arenaria*. This finding lead Berkeley to advance a theory that the crystalline style represents a reserve of oxygen and is a factor in the anaerobic respiration of mollusks. The theory is not supported by sufficient evidence and has not been accepted by the students of molluscan physiology.

The presence of oxidases in the styles of *Ostrea*

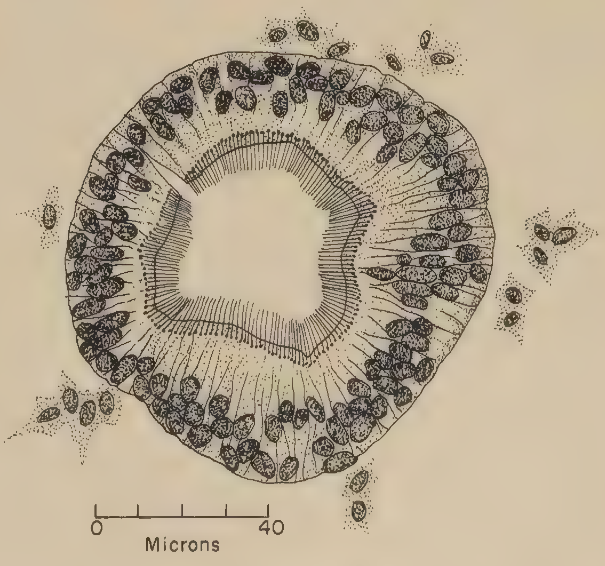

FIGURE 209.—Cross section of the duct leading to the digestive diverticula. Kahle, hematoxylin-eosin.

was confirmed by Yonge (1926a) and Graham (1931a, 1931b). The enzymes were obtained by grinding the styles with sand and extracting for 2 to 3 days in distilled water with a small amount of toluol as an antiseptic. For testing, the 5-ml. samples of 1 percent extract of style were treated with 2 ml. of hydrogen peroxide and 12 drops of 1 percent pyrogallol. After 5 minutes the sample turned dark red-brown. The extract produced color even in the absence of hydrogen peroxide, indicating the presence of a complete oxidase system. Reactions with guaiacum and 2 percent hydroquinine were less pronounced than with pyrogallol.

Sawano (1929) reported the presence of butyrase, an enzyme that clots milk, in the styles of *O. circumpicta* but his observation remains unconfirmed.

Extracts of digestive diverticula contain a large array of sucroclastic enzymes which act on starch, glycogen, sucrose, maltose, lactose, raffinose, and on some glucosides. The amylase, which converts starch into dextrin and dextrin into maltose, is present both in the style and in the digestive diverticula of the oyster. It has, however, different optima; the style amylase acts best at pH 6.0, whereas the enzyme from the diverticula has an optimum at pH 6.4 (Sawano. 1929).

The proteolytic enzyme of *O. edulis* is absent in the gut but can be found in the extract of the diverticula. It acts very slowly and has two pH optima at 3.7 and 8.5 when casein is used as a substrate. With gelatin the optima are 4.1 and 8.5.

Cellulase, the enzyme which hydrolizes cellulose, has not been found in the digestive extracts of the oyster. It must be assumed, therefore, that the oysters are unable to digest cellulose. The possibility is not excluded, however, that this enzyme may be present in the bacteria and fungi which happen to be in the gut. The presence of cellulase in mollusks has been established for the gastropods *Helix* and *Linnaea* and for the wood boring bivalve *Teredo.*

Fats are hydrolized to fatty acids and alcohols by the action of lipase. Yonge (1926a) demonstrated the presence of this enzyme by feeding the oysters an emulsion of olive oil stained red with Nile blue sulphate and watching the change of red color into blue as the digestion proceeded. Oil is ingested by phagocytes and is carried by them through the tissues, the gradual change of color serving as an index of the action of lipase. From the observation that the droplets of oil found free in the stomach retain the red color, Yonge deduced that free lipase is absent in the gastric juice. These findings are contradicted by the observations of George (1952) who showed that in *C. virginica* and in *Mytilus* the hydrolysis of neutral fats takes place extracellularly in the stomachs and that lipase can be extracted from the crystalline style. According to his observations, droplets of olive or peanut oil stained scarlet red with Sudan I or Sudan III are not deposited in the tissues. It is known that in mammals and birds the stained fat may be stored in the bodies (Gage and Fish, 1924). Several possibilities may be considered: (a) that the stained fat is rapidly metabolized; (b) that it may be deposited in connective tissue in minute quantities undetectable under the light microscope; and (c) that the mollusks are unable to utilize the peanut and olive oil because of the differences between the fatty acids of these oils and the unsaturated fatty acid of their natural food. So far no experimental evidence has been presented in support of any of these possibilities (George, 1952) and further studies of the problem of fat digestion in bivalves are needed.

pH CONTENT OF GUT AND STOMACH

The digestive fluids found in the alimentary tract are acid. The most acid conditions exist in the stomach (average pH 5.5) due to the dissolution of the crystalline style, which has a pH of 5.2 (5.4 in starved animals), and, according to Yonge (1926a), is the most acid substance in the gut. In the absence of the style the pH of the stomach fluids increases. This has been demonstrated on oysters with clamped shells, kept for 6 days out of water. Under these conditions the pH of the stomach rose from 5.67 to 6.14 while the pH of the liquid in the mantle cavity decreased due to the accumulation of carbon dioxide from 6.7 to 6.14. It is significant that the acidity in the stomach caused by the dissolution of the style approximates the optimum (pH 5.9) for the action of the style's amylase. The pattern of pH differences in various parts of the alimentary tracts as shown by Yonge is as follows: esophagus 5.6–6.0; stomach 5.4–5.6; style 5.2; midgut 5.5–6.0; rectum 5.8–6.3. The pH of the extracts of the styles of *C. virginica*, determined by placing the styles across the one-drop electrode, was found by Dean (1958) to vary between 5.8 and 6.0.

Extracts of the digestive diverticula of *O. edulis* have pH values from 5.6 to 5.9; the variations are probably associated with the resting and active stages of digestion. The styles of *C. virginica* contain a heat-labile substance, probably an enzyme, which has the ability to attack certain algal cells only during the dissolution of the style or within a very short period after the dissolution. This has been reported by Dean (1958) who observed rapid disintegration of *Cryptomonas* cells in buffered sea water (pH 6.0) containing style extract. *Monochrysis* sp. were immobilized by the extract while *Isochrysis* sp. were not affected and were able to swim near or even touch the style. Dean thinks the "enzyme" may be a protease, lipase, or amylase. The observed results may be interpreted as the differences in the resistance to digestion by different species of algae used by the oyster as food.

It has been pointed out in support of the importance of extracellular digestion that fragments of partially disintegrated large diatoms (*Coscinodiscus*, *Melosira*, *Skeletonema*) are frequently found in the stomachs of *C. virginica* (Nelson, 1934), but the question of the significance of extracellular digestion in bivalves has not been settled. Weak proteolytic action was found in the stomach of the giant clam, *Tridacna*, the pearl oyster, *Pinctada* (Mansour-Bek, 1946, 1948), and in the crystalline style extract of *C. virginica* (Chestnut, 1949) and strong amylolytic activity in the stomach of the oyster was demonstrated by a number of investigators. Oysters apparently have a great capacity to utilize materials rich in carbohydrates.

ABSORPTION OF FOOD BY GILLS AND MANTLE

The idea that the exposed surfaces of bivalves, particularly the gills, palps, and mantle, absorb the organic matter dissolved in sea water (Ranson, 1926, 1927) is not substantiated by experimental evidence. In experiments with *O. edulis* Yonge (1928) has shown that the oyster absorbs glucose from the water but that this absorption takes place through the alimentary canal and digestive diverticula. No absorption was recorded in the animals in which the access of water to the esophagus was prevented by stuffing the mouth with wax plugs. Glucose may be absorbed, however, by the phagocytes which accumulate on the surface of the mantle. The results of Yonge's observations were confirmed by Koller (1930) in his experiments with *Mytilus edulis* and *Mya arenaria.*

Since phagocytes normally aggregate on the surface of the mantle and gills, it is possible that the oyster may absorb the substances present in the surrounding media by means of these wandering cells. Yonge admits this possibility in the case of oysters fed iron saccharate, and I observed that the particles of iron oxide added to the water in which I kept *C. virginica* were ingested by the phagocytes of the gills and transported to the deeper parts of the body.

FOOD AND FEEDING

The study of food of the oyster has attracted the attention of many investigators who examined the stomach contents and recorded the variety of organisms found in it. One of the earliest observations was made more than a century and a half ago by Reade (1844, 1846), who was "induced" to examine the contents of the stomach of British oysters and the "well known ciliary currents in the fringes of the oyster." His curiosity was well satisfied, for he found "myriads of living nomads, the *Vibrio* also in great abundance and activity, and swarms of a conglomerate and ciliated living organism, which may be named *Volvox ostrearius*, somewhat resembling the *Volvox globator*, but so extremely delicate a structure, that it must be slightly charred to be rendered permanently visible." He listed also a number of common diatoms, silicoflagellates, and desmids which he called "Infusoria." It is impossible to guess the true identities of the "*Vibrio*" and "*Volvox.*"

Since the oyster is a filter feeder it is natural to expect that the contents of its alimentary canal would reflect the material suspended in water. Many of the investigators were unduly impressed by the occurrence of one or several species in the stomach and because of their abundance considered them to be of primary importance in the oyster diet. Opinions based on such examinations referred to the following forms found in the European oyster as important food materials: *Navicula fusiformis* v. *ostreari*, Grün. (Puységur, 1884); desmids, minute animals, and dead organic matter (Hoek, 1883); bottom diatoms *Nitzschia punctata*, *N. acuminata*, *N. sigma*, *Grammatophora oceanica*, and *Diploneis bombus* var. *densestriata*, the latter species being considered of special importance for fattening of oysters (Hinard, 1923). American biologists made similar observations in *C. virginica.* McCrady (1874) concluded that "diatoms and spores of algae" constitute the food of Carolina oysters; Lotsy (1893) found that in the James River, Va., "oyster lives almost exclusively on diatoms"; according to Smeltz (1898), the natural food of Florida oysters "can be supplemented by . . . the pollen of our pine trees and the bloom of our palmetto", (p. 307) but no evidence was presented that pollens were found in the stomachs or that they can be digested by the oysters. The flourishing and fattening of oysters in Delaware Bay was attributed by Nelson (1947) to the abundance of the diatom *Skeletonema,* which he called "the most valuable of all diatoms in the food of oysters in New Jersey waters." In an earlier paper (1923b) and in the Report of the New Jersey Agricultural Experiment Station for the year 1924 (Nelson, 1925), he emphasized the significance of nannoplankton which "comprise by far the largest part of the food of the oyster" and at times is composed of small flagellates and other minute forms which may comprise 80 to 90 percent of the stomach's contents. Since no plankton analysis was made by Nelson of the Delaware Bay water at the time of the *Skeletonema* bloom, the conclusion that the species is "the most valuable" requires corroboration.

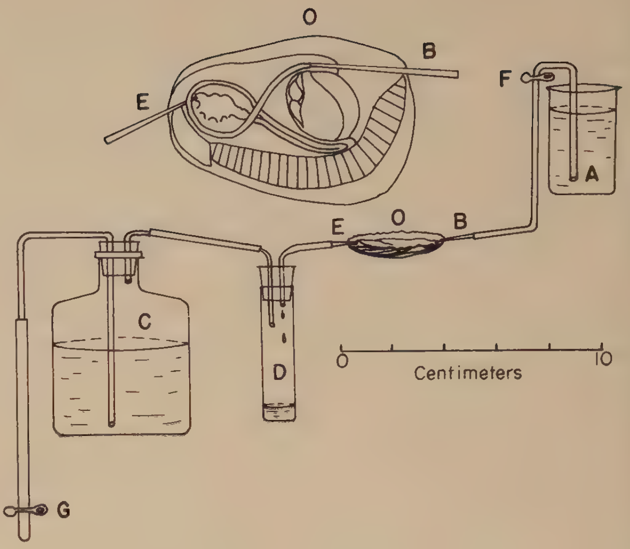

FIGURE 210.—Moore's method for washing out the stomach and intestinal content of the oyster. A—reservoir with sea water; B—canule inserted in the rectum; C—aspirator; D—collecting vessel; E—canule inserted into mouth of the oyster; F—siphon of the reservoir; G—siphon of the aspirator; O—oyster. Upper insert O—shows the details of the method of inserting canules B and E into the oyster.

Moore (1910) found that eight species of diatoms constituted 98 percent of the total amount of food in the alimentary tract of Texas oysters and that organic detritus also might play an important part in nutrition. Experimental studies of the feeding of oysters made by Martin (1927b) showed no significant differences between the average increases in size of young oysters which were fed pure cultures of the diatoms—*Nitzschia palea, Amphora coffeaeformis, Nitzschia poleaceae, Amphora coffeaeformis* var. *lineata,* and one species of green alga, *Gloeocystis vesiculosa.* No check was made on the amount of food added to the water and the experiment lasted only 4 weeks. Water was changed only once during this period. Because of the obvious deficiencies in the experimental technique no definite conclusions could be made from these observations. Martin also suggested that zoospores of *Enteromorpha* and other algae (*Ulva, Monostroma, Ectocarpus,* and *Pylaiella*) form an important element in the food of plankton eaters (Martin, 1927a). A comprehensive investigation of the food of the European oyster was made by Savage (1925), whose work remains the most valuable contribution to the study of the problem. He used Moore's (1910) method of washing the entire alimentary canal; this technique is diagrammatically shown in figure 210. Two canules are introduced, one into the anus, B, and the wider one, E, into the mouth. Rubber tubing connects the anal canule with the siphon F inserted in glass container A filled with sea water. The oral canule leads to a small collecting vessel D, which is connected to the aspirator bottle C. By regulating the flow of water from the aspirator C the alimentary canal may be washed out without damaging the digestive tract. The volume of the collected material is measured and the collected microorganisms identified and counted. By this method Savage (1925) sampled at regular intervals the stomach contents of British oysters and analyzed throughout the year the seasonal fluctuations in the abundance of different species of algae. He considered that the following diatoms were the most important food items of the British (Oxford) oysters: *Nitzschiella parva, Pleurosigma* sp., *Coscinodiscus* sp., *Rhizosolenia* sp., and *Melosira* sp. The most significant conclusion made by Savage is that the greater part of the food found in the oysters examined by him consisted of organic detritus and that "the animate food (i.e., living microorganisms) never exceeded 10 percent of the total" (by volume). He also advanced a hypothesis which, however, lacks experimental confirmation, that growth of Oxford oysters was due mainly to the inanimate food (detritus) and that fattening was caused by diatoms (*Nitzschiella longissima* f. *parva*). He found no evidence of selection of food by the oysters and commented that the actively feeding oyster appears to ingest anything that it can capture.

The extreme view that phytoplankton is of no direct significance as food of *O. edulis* in Danish water was expressed by Blegvad (1914), who classified this mollusk as a "pure detritus eater." Phytoplankton, according to his view, contributes to the food only as part of the detritus after the death of the algae.

Petersen and Jensen (1911) attributed great importance to eel grass, *Zostera*, as a possible source of food for bottom organisms. On the basis of their observations Spärck (1926) experimented with *O. edulis*, which he kept in a tank with sea water to which he added a liberal supply of old brown *Zostera*. Examination of the stomach contents of these oysters showed many species of flagellates and some *Zostera* detritus, but the quantity of the latter was by no means greater than in the oysters from the natural bottoms in the fjord. Decaying *Zostera* probably fertilized the water and stimulated the growth of the plankton. Danish investigators emphasized the fact that pentosan released from the decaying *Zostera* is a principal source of organic food for bottom invertebrates. The substance is apparently useless to oysters because they are unable to digest it, as has been shown by Yonge's experiments (1926a). The question of the extent of utilization by the oyster of the organic detritus which is always present in its natural environment has not yet been settled.

Naked flagellates and infusoria are frequently found in the contents of the alimentary tract. Under the influence of gastric fluids these forms are rapidly destroyed and, therefore, cannot be enumerated with any degree of certainty. The same problem applies to the bacteria which reach the alimentary canal. That they may play a considerable role in the feeding of lamellibranchs is indicated by the experiments of ZoBell and Landon (1937), and ZoBell and Feltham (1938), with the California mussel, which was fed known amounts of red coccus and a spore-forming bacillus. Within 3 hours the mussel removed about 200 million bacteria per 1 ml. of water. The microorganisms were actually ingested and after 6 hours disappeared from the digestive tract. In 9 months the mussels which were fed red coccus gained an average of 12.4 percent, the bacillus fed animals gained 9.7 percent, and the fasting mussels, kept as controls, lost about 6.8 percent. These experiments suggest an explanation of the observations by Kincaid (1938) that oysters kept for several months in water with nothing to feed on except bacteria appeared to be normal and even increased their glycogen content. Kincaid's experiment should, of course, be repeated and the question of the role of bacteria should be adequately studied before a conclusion can be made of their significance in the feeding of oysters and other bivalves.

By feeding the oyster known concentrations of coliform bacteria, Galtsoff and Arcisz (1954) found that 15 minutes after the start of addition of the culture the two oysters retained from 21 to 49 percent of *Escherischia coli* available in sea water. The accumulation of bacteria soon reached the point at which no more microorganisms were retained and the effluent leaving the oysters contained more *E. coli* than the surrounding water. Retention and elimination of microorganisms are probably associated with the secretion and discharge of mucus by the gill epithelium. These results confirm the previous observations by Galtsoff (1928) that over 50 percent of the bacteria pass through the gills and that only a fraction of their total number is retained.

The organisms found in the stomach of the oyster reflect the composition of plankton and nannoplankton present in the surrounding water. Selection is made primarily by the size and shape of food particles, although the ability of the oyster to discriminate between two suspensions of microorganisms of different colors but of the same size was suggested by Loosanoff's experiments (1949). A more detailed study should be made, however, before the discriminating ability of the oyster is confirmed.

There are several weaknesses common to all the studies on the feeding of oysters. The conclusions are based on examinations of the contents of the stomach and composition of feces without giving proper consideration to the nutritive value of different forms and their digestibility. The simple test of feeding the oysters inert materials such as carmine powder, carborundum, clay, pulverized williamite, and colloidal carbon would show that these undigestible materials, if fed gradually and not in excessive quantities, are swallowed and pass through the digestive tract. The fluorescent mineral williamite, which I used extensively in my studies, is particularly suitable for this purpose because it permits easy detection of the most minute granules of the mineral inside the intestinal tract or in the feces when illuminated by ultraviolet light. The fact that some of the microorganisms found in the stomach are not destroyed and can be recaptured alive in the feces has been known for a long time. The dinoflagellate *Prorocentrum micans* was seen by Blegvad (1914, p. 47) to pass unharmed. Living *Chlorella* and *Nitzschia closterium* given to *C. virginica* in large quantities can be recovered alive from the feces and recultured (Loosanoff and Engle, 1947). In studies of the effect of feeding oysters in the laboratory I frequently used a light suspension of Fleishmann's yeast, and observed that such a large number of yeast cells passed undigested that the feces acquired a milky color. Thus, the presence of an organism or its remnants in the alimentary tract in itself is not a proof that it is being used by the oyster as food and that it has nutritive value. Neither the enumeration of the organisms found in the stomach nor the determination of their volume gives satisfactory quantitative data. It is at present impossible to judge whether, for instance, one cell of *Coscinodiscus* equals or differs in nutritive value from a single cell of *Pleurosigma*, *Skeletonema*, *Nitzschia*, or other forms. Information is lacking about the caloric value and chemical composition of various forms and, therefore, it is impossible to determine the number that should satisfy the energy requirements of the oyster.

Through trial and error oyster growers know that certain grounds in their possession are particularly suitable either for the growth or for fattening and conditioning of oysters for market. Sometimes a great difference in the productive capacity of grounds may be found in the two areas located a short distance apart. In an ecological survey of the bottom it is relatively easy to detect conditions which are unsuitable for growth. It is, however, impossible at present to evaluate the potential productivity on the bottom because of the inadequacy of our knowledge of the nutrition of the oyster.

ARTIFICIAL FEEDING

So far only a few experiments on artificial feeding reported in the literature were successful in producing an increase in the weight of the oysters. As a rule oysters kept in the laboratory show lack of nutrition and die sooner or later. Better results may be obtained by keeping them in large outdoor tanks adequately supplied with sea water which has not been stored for any length of time. Experiments by Martin (1927a, 1927b) in feeding oysters with pure cultures of plankton forms resulted in very poor growth. Spärck (1926), experimenting with *Zostera* as a potential food for the European oyster, emphasized the fact that oysters "may thrive, increase in size and even spawn in very small limited water volumes without any renewal of water worth mentioning." Such conditions occur in the Norwegian oyster basins and in the French "parks" which, however, must contain "some source producing nourishment in sufficient quality and quantity." This material presumably may derive from the organic detritus. He also reports that in his experiments the "development of bacteria did not seem in any way to hurt the oyster, rather the opposite."

A unique experiment, unfortunately not well known to biologists, was made by Gavard (1927, quoted from Korringa, 1949) in Algiers. He fed the oysters an artificial detritus prepared from animal and plant material and obtained an increase of 15 kg. per 1,000 oysters per season. Korringa states that these results demonstrate the ability of the oysters to grow without using living organisms as food. Without access to Gavard's original paper it is impossible to judge if the detritus was directly consumed by the oysters as food or whether it stimulated the growth of bacteria and nannoplankton.

Artificial enrichment of sea water by adding commercial fertilizers at one time seemed to be a simple answer to the problem of providing increased food supply to the oyster. To test the idea a series of experiments was conducted in the Bureau of Commercial Fisheries Biological Laboratory at Milford, Conn., which resulted in the interesting discovery that an excessive concentration of microorganisms (*Chlorella* sp., *Nitzschia closterium*, *Prorocentrum triangulatum*, *Euglena viridis*) adversely affects the feeding of oysters

(Loosanoff and Engle, 1947). A large-scale "natural" experiment along the same line took place in Great South Bay where unbalanced fertilization of sea water by manure from the duck farms located along the banks of the bay boosted such reproduction of *Chlorella*-like organisms that the heretofore prosperous shellfish industry of the bay suffered a serious setback (Redfield, 1952).

Nelson (1934) made a series of tests of several substances as artificial foods for oysters. He used corn starch, ground alfalfa, soybean meal, and ground meat of the king crab. It is not clear in his report if the criterion of results was the weight of the oyster meat. Nelson states that only with corn starch "was any success obtained." The details of these experiments have not been disclosed.

In spite of doubtful results the artificial feeding of oysters appears to be a definite possibliity which should be carefully investigated. Since oysters are able to absorb glucose dissolved in sea water (Yonge, 1928), it seems desirable to explore more thoroughly this method of feeding. Furthermore, the diet of the oyster and the nutritive value of different diatoms and flagellates should be investigated together with the methods of their cultivation. It is reasonable to expect that certain forms richer in protein, may be more useful for obtaining better growth of oysters; others, richer in carbohydrates, may prove more valuable for their fattening. Research along these lines offers many interesting possibilities that may prove useful in the artificial culture of oysters.

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CHAPTER XI

THE CIRCULATORY SYSTEM AND BLOOD

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A heart, arteries, veins, and open sinuses form the circulatory system of oysters and other bivalves. The sinuses, or lacunae, are irregular spaces of varying size in the tissues and have no walls of their own other than the surrounding connective tissue. They are interposed between small arteries and veins and function in place of the capillaries of vertebrates. Blood cells are not confined to the vessels; they wander throughout the tissues, aggregating in the sinuses. A large number of them accumulate on the surface of the mantle and gills and are discarded. Diapedesis, i.e., slow bleeding through the surface of the body, is a continuous and normal process which is accelerated by adverse conditions, by injuries to the tissues, and by removal of an oyster from its shell.

The open sinuses within the circulatory system present a mechanical puzzle. It is difficult to visualize how the pressure of the systolic contraction forces the blood to leave the open spaces and enter the venal system, which has no valves, go through a complex net of branchial vessels and finally enter the heart. To a great extent the mechanical deficiency of the circulatory system is compensated by the pulsating vessels of the mantle and by the contractions of two accessory hearts on the walls of the cloacal chamber. The pulsations of these organs are independent of the beating of the principal heart, and their primary function is to oscillate the blood within the pallial sinuses.

THE PERICARDIUM

The heart is located in the pericardium, a thin-walled chamber between the visceral mass and the adductor muscle (fig. 71). In a live oyster the location of the heart is indicated by the throbbing of the wall of the pericardium on the left side. Here the pericardium wall lies directly under the shell. On the right side the promyal chamber extends down over the heart region and the mantle separates the pericardium wall from the shell.

The cavity in which the heart is lodged is slightly asymmetrical; on the right side it extends farther along the anterior part of the adductor muscle than on the left. The pericardium is large enough to accommodate the heart and to retain a supply of the fluid in which the heart is bathed. The volume of the pericardium can be measured by the following method. A solution of plastic or a thin mixture of plaster of paris is poured into the pericardium from which the heart has been removed; after the material has set, the plaster molds are waterproofed by immersing them in a hot mixture of beeswax, rosin, and turpentine. The volumes are measured by displacement. In an adult *Crassostrea virginica* about 12 to 14 cm. in height, the capacity of the pericardium varied from 2.4 to 2.7 ml.; approximately the same volume of blood and pericardial fluid could be withdrawn from the cavity by hypodermic syringe.

Two reno-pericardial canals open on the right and left side of the ventro-posterior wall of the pericardium and provide direct communication with the excretory system (see: ch. XII). The wall of the pericardium is formed of connective tissue similar to that in the mantle; the tissue is well supplied with blood vessels, blood sinuses (figs. 211 and 212), and branches of the cardiac nerve (fig. 213). The epithelium lining of the side

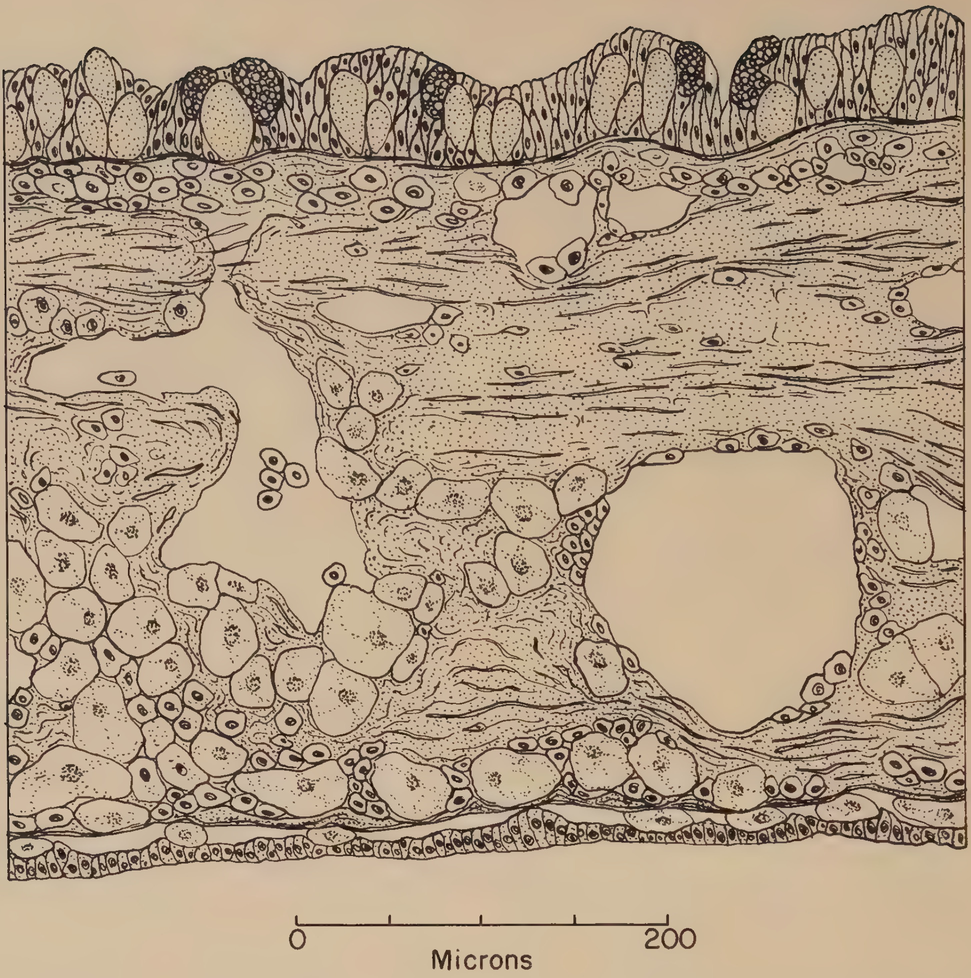

FIGURE 211.—Transverse section of the pericardium wall of *C. virginica*. Surface epithelium is rich in mucous (light) and eosinophilic cells (dark granules). Large vein (right) and blood sinus (left). The epithelium of the inner sides (lower side of the drawing) faces the heart. Bouin, Mallory triple stain.

facing the heart consists of small flattened cells and a few scattered eosinophilic and mucous cells; on the opposite side, facing the shell, the pericardium wall is covered with large columnar epithelial cells with oval nuclei and many eosinophilic and mucous cells. Basal membrane on the upper side of the wall is well developed.

THE HEART

The three-chambered heart is suspended obliquely in the pericardium and is held by the root of the aorta on one side and by the common efferent veins on the other. The ventricle is larger and bulkier than the two auricles; a constriction between the ventricle and auricles marks the partitition between them (fig. 214). The auricles are darkened by pigment cells in their walls. The degree of pigmentation varies from light brown to almost black. The ventricle is a pear-shaped structure slightly constricted along the middle. Its walls are formed by thick bundles of nonstriated muscle fibers which traverse the ventricular cavity and incompletely divide it into two chambers.

In the majority of bivalves the rectum passes through the heart, but in the oyster the rectum lies behind the heart (fig. 71).

The fibers of the heart muscle cross one another in many directions, frequently branch and anastomose, and are surrounded by delicate connective tissue. In general the muscle tissue has a spongy appearance (fig. 215). In the ventricle the muscle fibers are thicker and stronger than in the auricles.

The wall of the ventricle and the septum between the two parts of the heart are formed by a

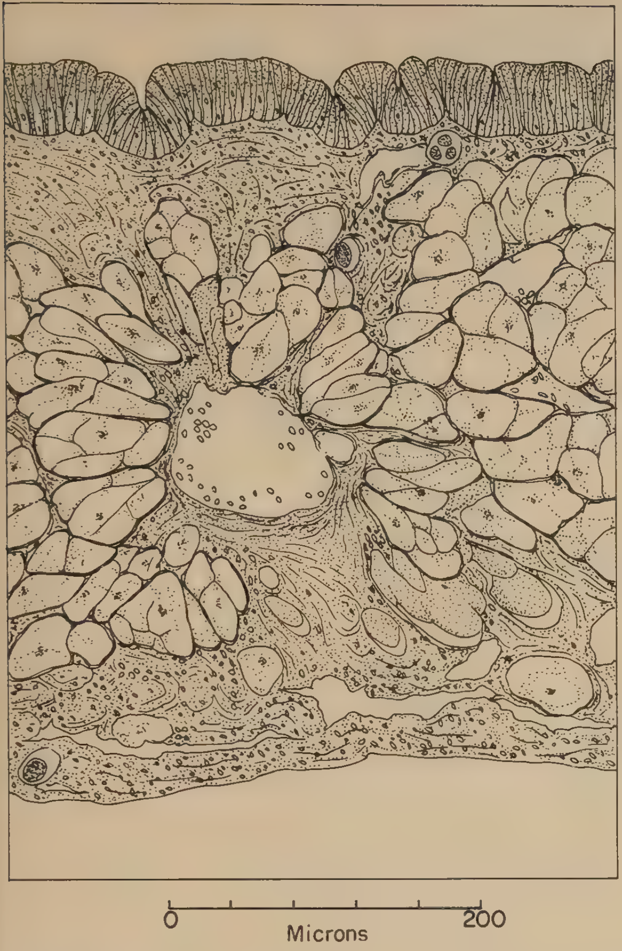

FIGURE 212.—Transverse section of a portion of the pericardium wall of *C. virginica* with an artery surrounded by large vesicular cells. NEMATOPSIS cysts on upper right and lower left sides. Bouin, hematoxylin-eosin.

framework of muscle fibers and connective tissue cells forming an irregular trabecular structure (fig. 216), with amoebocytes in the spaces between the fibers and in the connective tissue. The outer surface of the ventricle is covered with epithelium of a single layer of flat and thin cells with conspicuous nuclei.

The walls of the auricles, thinner and lighter than those of the ventricle, also form a trabecular framework supported by connective tissue (fig. 217). Amoebocytes are numerous between the connective tissue cells and along the muscle fibers. On the outside the auricles are covered with tall columnar epithelium which contains many glandular and dark pigment cells; this epithelium constitutes a part of the excretory system in bivalves (Franc, 1960, p. 2016). Neither the ventricle nor the auricles has an inner epithelial lining.

The movement of blood from the auricles to the ventricle is controlled by the two auriculoventricular valves which appear as circular bands of tissue surrounding small openings (fig. 218). In longitudinal section the auriculo-ventricular valve (fig. 219) resembles a convoluted cylindrical tube. The walls of the valves consist of several layers of muscle fibers arranged obliquely and supported by connective tissue. When the auricle (left part of fig. 219) contracts, blood is propelled into the ventricle (right portion of the figure), which in turn contracts, compressing the walls of the valves and forcing the blood forward into the aorta (not shown in fig. 219).

The heart is well supplied with ganglion cells and nerve fibers which end in the muscles. Preparations of heart tissue of *C. virginica* stained with methylene blue and examined in glycerin under oil immersion showed a great abundance of these elements (fig. 220). These observations support the findings of Suzuki (1934a, 1934b), who described the ganglion cells in the hearts of *Ostrea circumpicta* Pils., *O. gigas* Thunb., and *Pinctada martensi*. According to his data, the ganglion cells in these oysters are particularly abundant at the septum separating the auricles from the ventricle where they form a ring at the narrowest portion of the heart. Direct con-

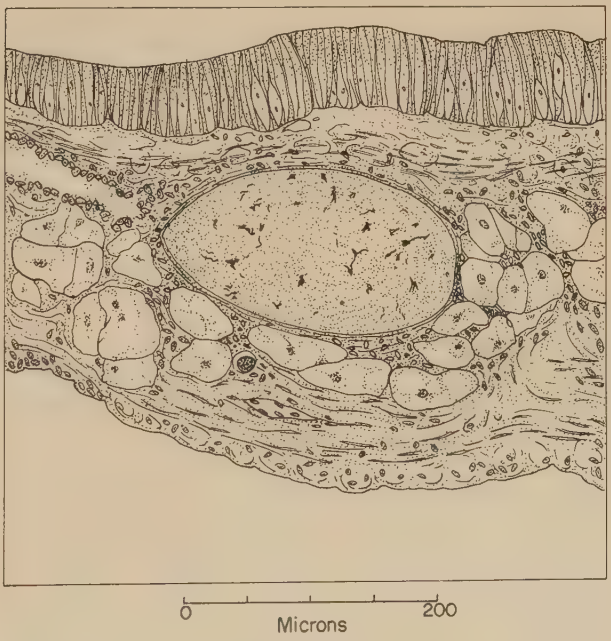

FIGURE 213.—Transverse section of the pericardium wall of *C. virginica* with the branch of the cardial nerve (cut at a slightly slanted angle). Bouin, hematoxylin-eosin.

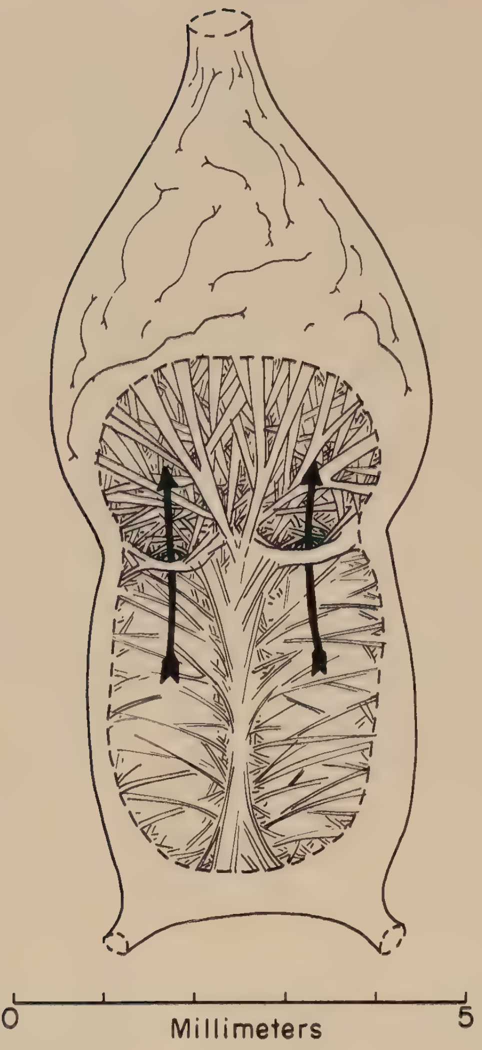

Figure 214.—Heart of the oyster *C. virginica* viewed from the ventro-anterior side. Part of the heart's wall was removed to show the auriculo-ventricular septum and the musculature of the heart. Upper part—ventricle and root of the aorta; lower part—two auricles and common efferent veins. Drawn from an unpreserved preparation.

nections between the nerve cells scattered in the heart muscle and nerve fibers entering the heart have not been demonstrated.

A summary of the results of many investigations of the innervation of the bivalve heart was given by Esser (1934), who denied the existence of the cardial ganglia in the heart of *Anodonta cygnea* and stated that the so-called nerve cells of the mollusk's myocardium have none of the typical features of the nerve cells. He thought that these cells were identical with certain amoebocytes of the blood of *Anodonta*. It is true that the amoebocytes found in the heart muscle of *C. virginica* have a certain similarity to the cells depicted by Esser. In structure and in general outline they differ, however, from the nerve cells and can be recognized in the preparations stained with methylene blue. Under high magnification the ganglia cells in the myocardium of *C. virginica* appear to be oval-shaped and bipolar (fig. 221) rather than unipolar as described by Suzuki (1934a) for *O. circumpicta*. Their cytoplasm contains granules deeply stained with methylene blue. Round granules of larger size distributed along the axis of the nerve are visible in vitally stained preparations (fig. 220). Similar structures are shown by Suzuki in his figure 4 (1934b) of the preparation of the heart muscle of the Japanese oyster (*C. gigas* and *O. circumpicta*). The nature of the granules is not known.

PHYSIOLOGY OF THE HEART

Contributions to the study of the physiology of the heart of bivalves have been made by Carlson in a series of papers published during the years 1903–09 (Carlson, 1903, 1905a, 1905b, 1905c, 1905d, 1906a, 1906b, 1906c, 1906d, 1907, 1909); by Ten Cate (1923a, 1923b, 1923c, 1929); Jullien (1935a, 1935b, 1935c, 1935d, 1936a, 1936b, 1936c); Jullien and Morin (1930, 1931a, 1931b); Jullien and Vincent (1938); Jullien, Vincent, Bouchet, and Vuillet (1938); Jullien, Vincent, Vuillet, and Bouchet (1939); Takatsuki (1927, 1929, 1933, 1934a, 1934b); Oka (1932); Suzuki (1934a, 1934b); Prosser (1940, 1942); and many others. The literature up to 1933 is adequately reviewed by Dubuisson (1933), and more recent investigations are summarized by Krijgsman and Divaris (1955). The studies cited above were made primarily on the fresh-water mussel *Anodonta*, on *Mytilus*, *Pecten*, and *Mya*. A relatively small number of observations were made on oyster heart.

AUTOMATISM OF HEART BEAT

Most of the experimental work on bivalve hearts has been done with excised preparations of the organ kept in a perfusion chamber supplied with the van't Hoff or Ringer solutions or with natural sea water. Few observations were made on the heart in situ.

An automatic rhythmical beating of the excised oyster heart continues for a long time if the heart is kept in an isotonic solution, preferably in sea water, at normal pH of about 8.0 or in the pericardial fluid, and the heart muscle is slightly

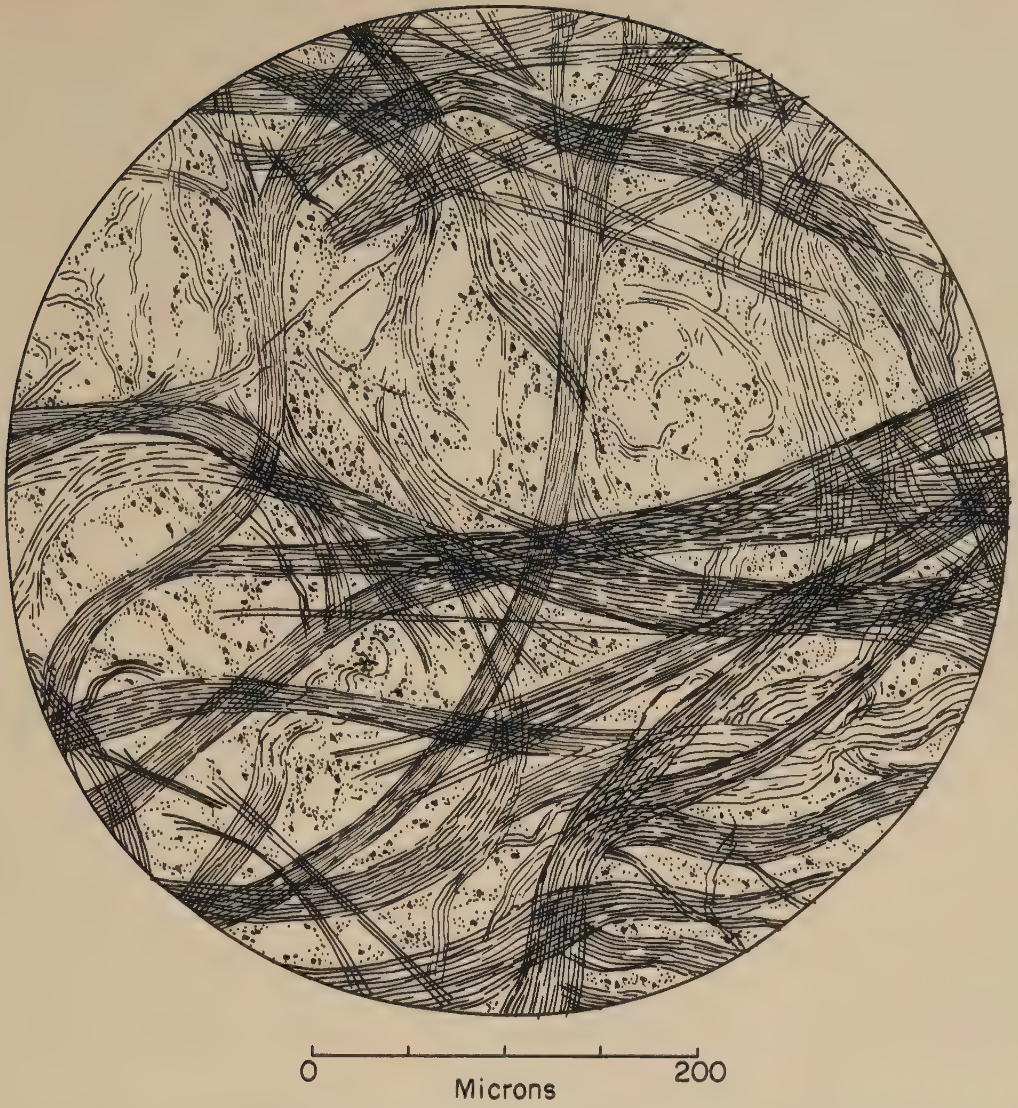

FIGURE 215.—Small piece of heart wall of *C. virginica* showing spongy appearance of muscles. Slightly compressed whole mount. Formalin, 5 percent, hematoxylin-eosin.

stretched by the pull of a light lever to which the aorta end of the ventricle is attached; the opposite end of the ventricle is tied to an immobilized glass rod. Gentle stretching is sufficient to provide the necessary stimulus. Takatsuki (1927) claimed that under these conditions the isolated heart of the Japanese oyster, *O. circumpicta*, may remain active for 16 days. Observations in the Woods Hole laboratory show that the excised hearts of *C. virginica* kept in sea water at room temperature continued to beat for 2 to 3 days, but the frequency and the amplitude of beat decreased noticeably after the first 24 hours.

The molluscan heart functions as a pressure pump which must develop considerable power to propel the blood through the circulatory system. The mechanical force during the systole is produced by the contraction of a trabecular wall made of many anastomosing fibers. This arrangement, also present in *O. edulis* (Jullien, 1935b), is shown in figures 214 and 215.

In a number of bivalves (*Anodonta*, *Mytilus*, *Ostrea*) the peristaltic wave in the ventricle starts at the posterior end and progresses forward (DeBoer, 1929; Ten Cate, 1923a, 1923b, 1923c). The contraction of the ventricle compresses the auriculo-ventricular valves (fig. 218) and prevents the reflux of blood into the auricles. There is an interval between the contractions of the ventricle and auricles which may be noticed by visual

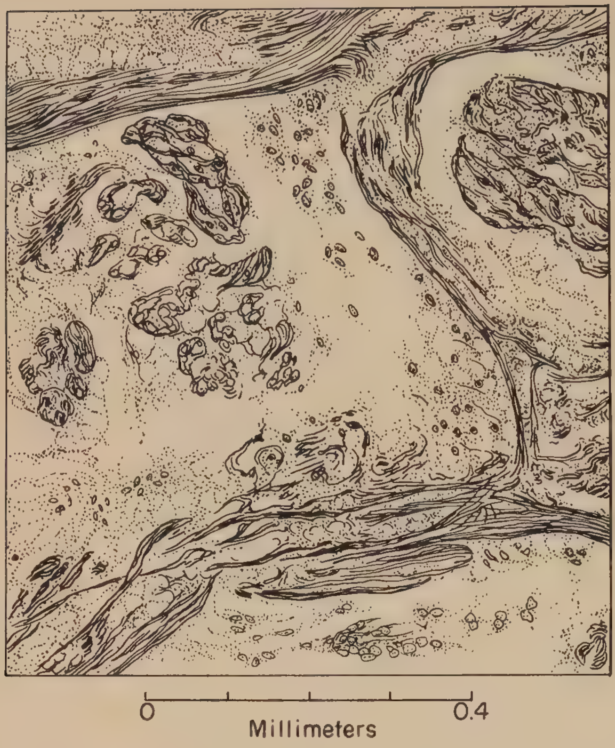

FIGURE 216.—Cross section through auriculo-ventricular septum of *C. virginica.* Formalin 5 percent, hematoxylin-eosin.

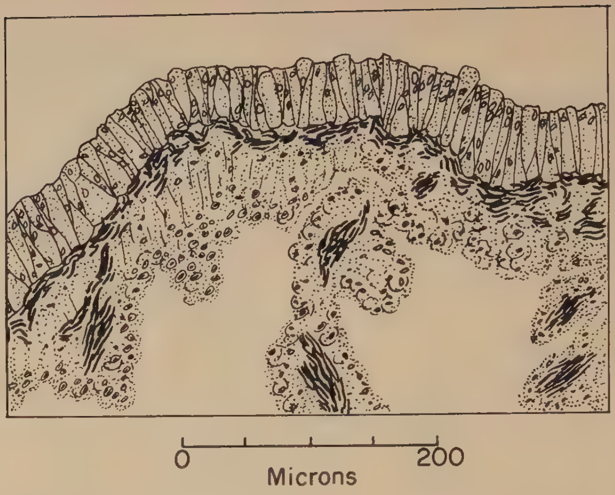

FIGURE 217.—Cross section of the wall of the auricle of *C. virginica.* Outside wall is covered with glandular epithelium. Bouin, hematoxylin-eosin.

inspection. The electrocardiogram of the oyster heart (*O. edulis*) published by Eiger (1913) shows that the interval is about 0.5 second. A similar condition in the heart of *C. virginica* was demonstrated on an electrocardiogram (fig. 222) made in the Bureau's shellfish laboratory by removing part of one valve and placing the electrodes on the pericardium wall and on the adjacent tissues. Action currents observed by Taylor and Walzl (1941) in the ventricle of the excised heart of *C. virginica* consist, according to their interpretation, of two components, a major diphasic wave preceding the contraction, and a slow wave at the time of contraction.

The refilling of the heart during the diastolic phase is dependent on pressure mechanism in the pericardium. Krijgsman and Divaris (1955) propose the following probable explanation which requires further corroboration. The change in the hydrostatic pressure in the pericardial chamber, caused by systolic contraction, is compensated by the expansion of the auricles. At the moment the ventricle starts to contract it exerts a suction which brings in blood through the reno-pericardial canal and venous system. Thus, the contraction of the ventricle automatically results in the expansion of the auricles. This interesting hypothesis may be corroborated by observations on hydrostatic pressure inside the heart and in the pericardial cavity and by motion pictures of the sequences of ventricular and auricular beat. To my knowledge these have not yet been made.

Observations on bivalve hearts in situ show that the ventricle and auricles alternately increase in size while they are being filled with blood. Both auricles of the oyster heart contract simultaneously (Skramlik, 1929).

Experimental evidence indicates that the autom-

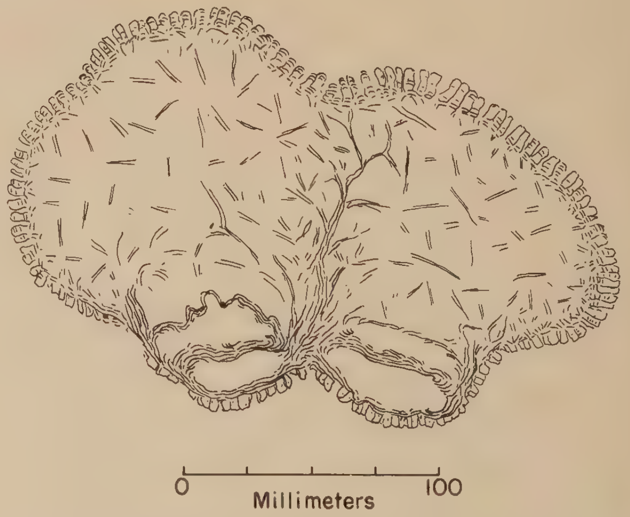

FIGURE 218.—Cross section of the heart at the auriculo-ventricular valves of *C. virginica.* Bouin, hematoxylin-eosin.

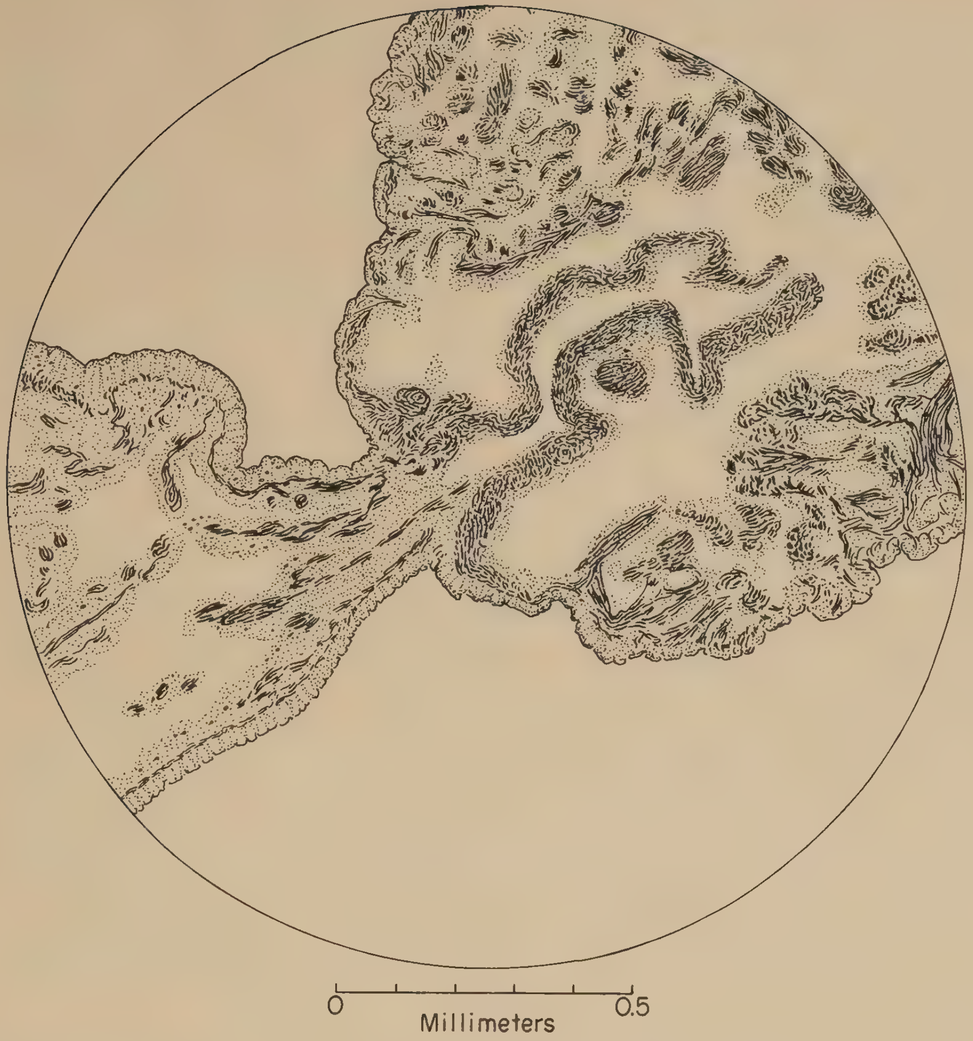

FIGURE 219.—Auriculo-ventricular valve of *C. virginica* seen in longitudinal section. Auricle on the left. Bouin, hematoxylin-eosin.

atism of the bivalve heart is of diffuse nature. Berthe and Petitfrère (1934a, 1934b) showed that contractions of the heart of *Anodonta* originate at any point of the ventricle whether it is observed in situ, or on isolated and even sectioned pieces. In these studies the authors used optical methods to record the beats of the hearts, which were fully submerged in Ringer solution or in *Anodonta* blood and were not stretched by writing levers. They found that such distension of the ventricle removed the asynchronism in automatic activity, increased the amplitude of the contraction, and diminished the rhythm. Jullien and Morin (1931a) reported that the pulsations in dissected strips of heart muscles of *O. edulis* continue for some time. One may conclude that the hearts of the oyster and other bivalve mollusks are myogenic, i.e., their intrinsic automatism originates in the muscular tissue. In the myogenic hearts of bivalves the beat can start at any point and the contraction can be local or involve the entire organ (Berthe and Petitfrère, 1934b). This type of activity differs from that of the neurogenic hearts, such as those of arthropods, in which the excitation wave of the beat originates from the nerve cells of the ganglia.

THE PACEMAKER SYSTEM

We know that the rhythmic activity of the hearts of bivalves originates in the heart itself and is not provoked by impulses from the central nervous system. Whether this automatism is produced by localized pacemakers or is a general property of all muscle fibers has not been adequate-

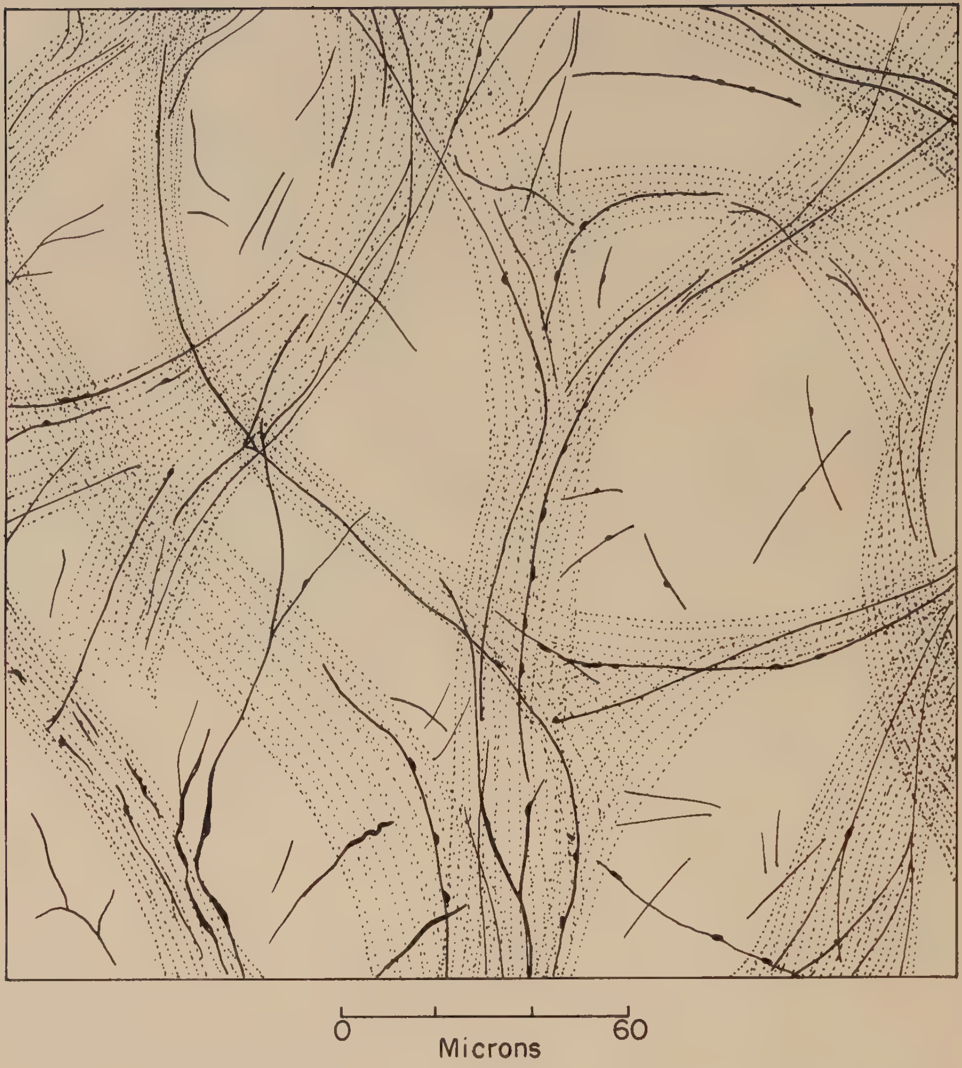

FIGURE 220.—Nerves in the heart muscle of *C. virginica* vitally stained in methylene blue. Glycerin-jelly.

ly studied. The presence of nerve cells in the heart has been confirmed for many bivalves, gastropods, nudibranchs, and cephalopods (Dogiel, 1877; Suzuki, 1934a, 1934b; Dubuisson, 1933). On the other hand several investigators deny the presence of nerve cells in the heart of mollusks and consider that connective tissue cells were mistakenly described as nerve cells (Krijgsman and Divaris, 1955). Motley (1933), Esser (1934), and Prosser (1940, 1942) were unable to find them in *Anodonta* and *Venus.* Inconsistencies in the results are probably due to the uncertainties encountered in staining nervous elements of the heart with the usual histological technique and frequent failures in using some brands of methylene blue.

It is known that in *Anodonta* and *Mytilus* the wave of ventricular contraction starts at the posterior end. Furthermore, by applying heating to various places of the hearts of *Anodonta, Unio,* and *Mytilus* DeBoer (1929) was able to show that warming the posterior part of the ventricle increases the beat frequency, whereas the heating of the anterior part has no effect (Krijgsman and Divaris, 1955). In the heart of a dying oyster (*O. edulis*), the aortic region continues to beat for a longer time than do the other parts of the organ; the isolated hearts seldom beat if the aorta is completely cut off from the preparation (Jullien and Morin, 1931a). This is also true for the longitudinal fragments of the heart, which continue to beat if they contain a piece of aorta. These observations seem to support the opinion that in most cases the bivalve heart possesses a diffuse myogenic pacemaker.

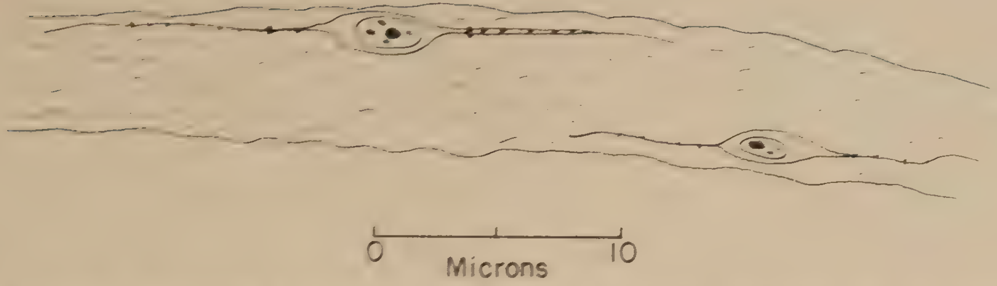

Figure 221.—Nerve cells in the heart muscle fiber of *C. virginica.* Methylene blue vital stain.

Pharmacological evidence of the effect of drugs on heart, described later (p. 252), and particularly the action of acetylcholine and the antagonism of curare to acethycholine, support the view that the pacemaker system in the oyster heart is of a diffuse myogenic nature.

METHODS OF STUDY OF HEART BEAT

In order to count the number of beats per unit of time a portion of the left valve must be removed without injury to the adductor muscle and the underlying tissue. The oyster is then kept in sea water at constant temperature, and the number of beats is recorded. The method was used by Federighi (1929) and by Koehring (1937), who drilled a small round window in the valve and with sharp scissors dissected the pericardium to expose the heart. These oysters lived for several weeks in running sea water in the laboratory of the Bureau of Commercial Fisheries at Woods Hole without noticeable ill effects.

Stauber (1940) modified the technique by cutting windows in both valves without injury to the pericardium wall and cementing them over with pieces of glass or cellophane. For observation the operated oysters were illuminated from underneath. In a few days both were covered by new shell and had to be replaced. Shell material that covered the window of the left side, where the pericardium wall touched the valve, probably spread from the adjacent areas of the mantle.

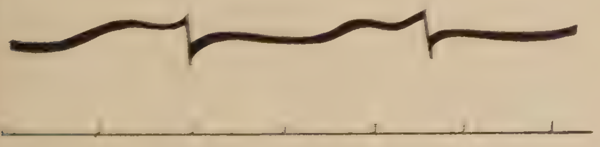

Figure 222.—Electrocardiogram of *C. virginica* taken in situ. A gentle wave corresponding to auricular contraction A precedes by approximately one-half second the contraction of the ventricle. Temperature 22.6 °C. Time intervals, 1 second.

Pulse records can be obtained without touching the heart itself by removing a portion of the valve, using the pericardium wall as a sphygmograph tambour, and providing a small stand made of light plastic to support one arm of the writing lever. The disadvantage of this method used in the shellfish laboratory at Woods Hole was that the heart became fatigued after several hours of recording.

There is another technique to study heart contraction in situ. The pericardium wall is exposed by cutting off the valve above the adductor muscle. A small S-shaped glass hook connecting the heart with the kymograph lever is placed under the auriculo-ventricular junction or under the ventricle. A silk thread tied to the upper part of the hook is connected to a writing lever, which is carefully balanced so that the tension on the heart does not exceed 100 mg. Care must be taken to adjust the tension so that the pull of the hook will not displace the heart from its normal position (fig. 223).

There will be a minimum of damage to the nervous system and adjacent organs if only part of the valve between the adductor muscle and the hinge is removed. This leaves the muscle itself intact, and only the pericardium wall is dissected to expose the heart. The oyster is kept in a known volume of water in a finger bowl, which is placed in a large crystallizing dish to permit the rapid change of water or of experimental solution without disturbing the setup. Temperature in the larger dish (not shown in figure 223) is thermostatically controlled at any desired degree. Under such conditions the beating of the heart continues for about 2 days.

The perfusion chamber method is frequently employed (fig. 224) in the pharmacological studies of the effects of drugs on bivalve hearts. In this method the heart is cut off at the levels of the auricles and the aorta, ligatures are applied at

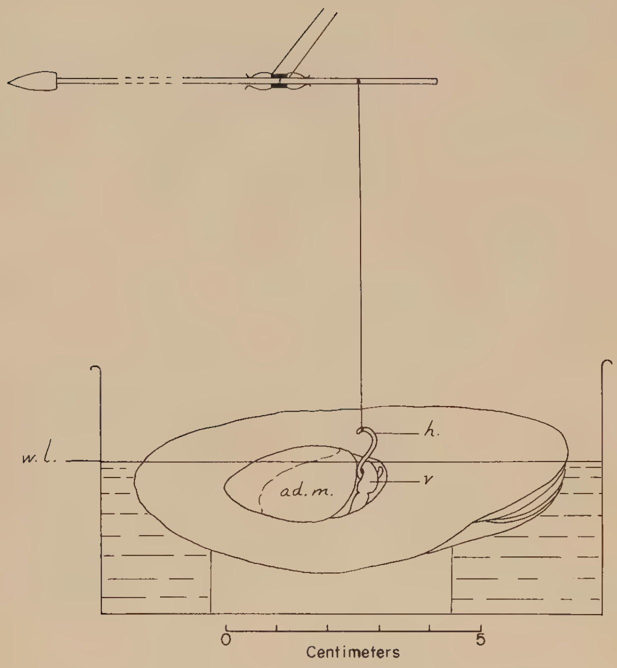

Figure 223.—Method of obtaining tracings of oyster heart in situ. ad.m.—adductor muscle; h.—glass hook under the ventricle, V; w.l.—water level. The upper valve has been removed, the pericardium dissected, and the oyster placed on a suitable base in a finger bowl.

both ends and the organ is placed in the perfusion chamber filled with sea water or with Ringer solution. The aorta end of the heart is connected to the writing lever, and the auricular end is attached to the base. The chamber is a glass tube about 2 cm. in diameter with an overflow arm near the top (fig. 224). The length of the tube may be adjusted to obtain the desired volume, usually 10 or 20 ml., between the bottom and the overflow. The liquid (perfusate) is delivered through an inlet A at the bottom; it fills the chamber to the level of the overflow and runs out through outlet B. The preparation may be aerated through a second glass tubing inserted in the bottom. Under this condition the heart remains alive and active for several days.

A very delicate technique to study the nerves which stimulate the oyster heart (*O. circumpicta*) was developed by Oka (1932). The preparation was made in the following manner: the shell was carefully cut off without any injury to the pericardial region and visceral ganglion; the greater part of the gills with the mantle were removed; the adductor muscle was dissected; and the oyster was fastened to a small board in the manner shown in figure 225. In this way the visceral ganglion with its nervous connection and the heart were exposed and made accessible for stimulation. The heart was kept in water, but the ganglion was exposed to air. The rhythm was recorded for the heart in situ and separately for the ventricle and two auricles. For the latter purpose the heart was cut at the auriculo-ventricular junction and the cut end tied with a silk thread. The free end was connected to a writing lever of a kymograph (upper right part of figure 225).

FREQUENCY OF BEAT

The heart beat of all bivalves is so greatly affected by the environment that reports of the rates of beat are of little value unless the conditions under which the observations were made are completely and accurately described. Frequency of heart beat increases with the rise of temperature and decreases with its fall. According to Federighi (1929), the response follows Arrhenius equation from which the so-called temperature coefficient (designated as μ) can be calculated, using the technique developed by Crozier. Discussion of temperature characteristics of biological processes in general and the application of the Arrhenius equation of the effect of temperature on chemical reactions to heart physiology is beyond the scope of this book. The reader interested in the problem is referred to Barnes' (1937) Textbook of general physiology, chapter XIII, or to chapter I in Crozier and Hoagland's (1934) Handbook of general experimental psychology. There is, however, serious reason to question the validity of

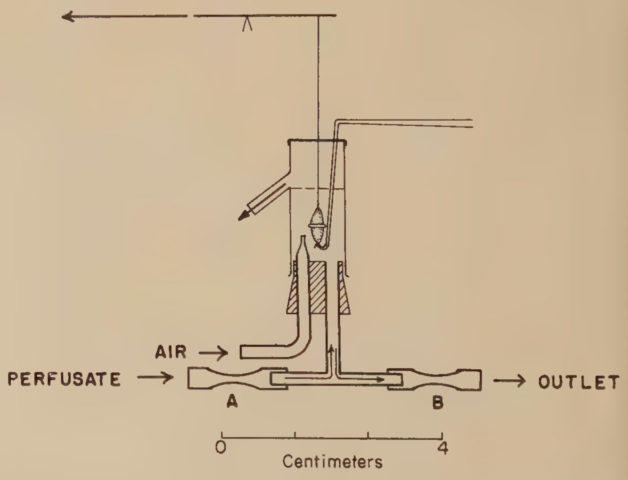

Figure 224.—Wait's perfusion chamber for recording the activity of an excised heart of mollusks. From Wait, 1943.

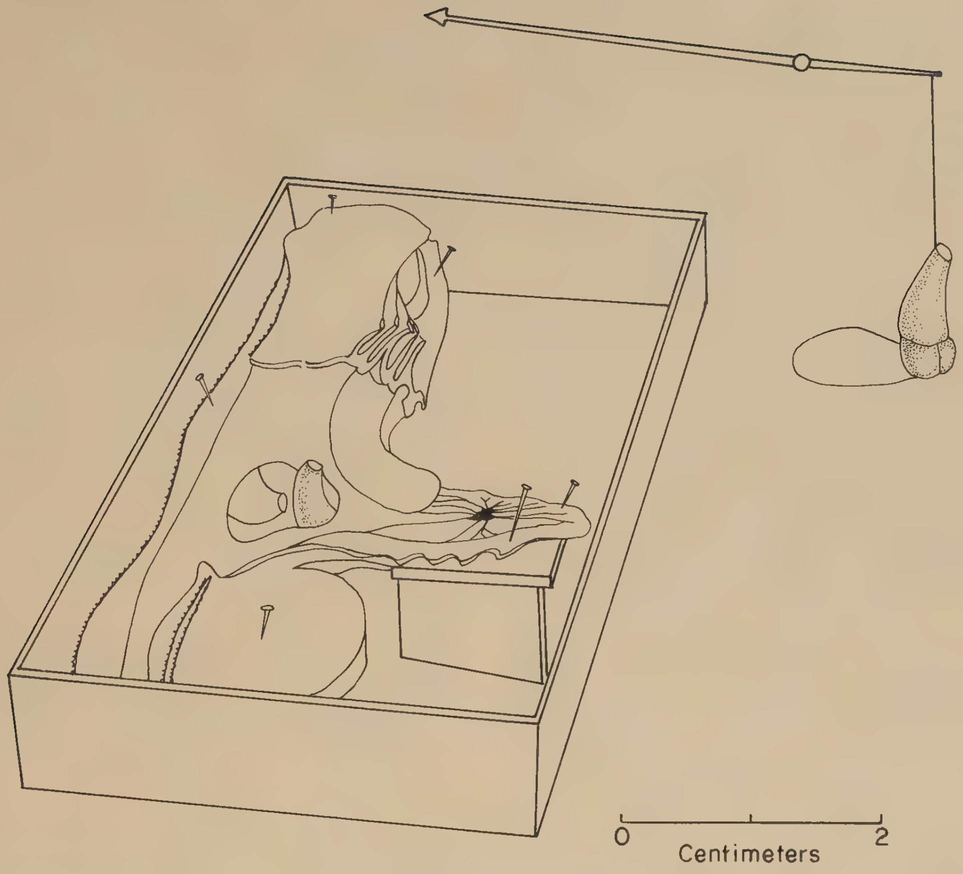

FIGURE 225.—Oka's method of exposing the visceral ganglion for study of heart stimulation in the oyster. Reproduced from Oka, 1932.

the underlying theory of temperature coefficients of biological reactions (Bělehrádek, 1935).

In experiments with *C. virginica* at the Woods Hole laboratory Federighi (1929) found the values of μ equal to 16,000 and 13,600. It is rather difficult to convert his data into conventional terms of number of beats per minute since his experimental results are presented only as plots of logarithms of the frequencies (time required for 10 beats) multiplied by 100 against the reciprocals of absolute temperature. At my request Federighi in a personal communication supplied excerpts from his laboratory notes which show the following rates:

Temperature	Beats per minute	Temperature	Beats per minute
25°	47	16°	21
21°–22°	35	10°	11

The rates appear to be much higher than those observed by others. In Federighi's experiments the upper critical temperature above which there was rapid decline in pulse rate was approximately 30° C.

In Koehring's (1937) observations on *C. virginica* the heart rate averaged 20 beats per minute at 20°. She found also that in the oysters with one valve completely removed the heart action was inhibited for several hours and there was no ciliary motion of the gill epithelium. Inhibition of the heart's activity when the shells are closed was reported by Stauber (1940) in oysters uninjured except for perforation of both valves. He found that the heart rhythm of *C. virginica* slowed down and became irregular when the oyster closed the valves. In some of the closed oysters the heart remained inactive for 2 to 3 minutes, then resumed beating at low frequencies of about two to three times per minute, only rarely exceeding six beats per minute at the temperature of 17.5° C. As the valves began to open, the heart beat increased to 14 to 16 times per minute. These results are in accord with observations on *Anodonta* and

Sphaerium (Cyclas) by Gartkiewicz (1926), who described the suppression of heart beat and of ciliary motion during the periods of shell closures. Because of the high transparency of the shell of *Sphaerium* the behavior of the heart of this mollusk could be observed under normal conditions. Gartkiewicz calls the inhibition of cardiac and ciliary activity the "sleep" of the bivalves. The cause of the heart's inhibition is not known; it is probable that in the case of *Sphaerium* the lowered pH of body fluids and the accumulation of carbon dioxide may have contributed to the suppression of cardiac activities. This, however, does not account for the observed temporary cessations of heart beats in the oysters and clams kept in sea water but with their valves partly removed. Apparently the stoppage associated with the contraction of the adductor muscle was due to inhibition originated from the nervous system.

The heart beat in *O. circumpicta* of Japan reaches a maximum of 30 beats per minute at 35° C. and slows down to three beats per minute at 5° C. and to 14 at 40° C. No heart action was recorded by Takatsuki (1927) at 0° and at 45° C. Climatic conditions apparently influence the heart rhythm since it was shown by the same author (Takatsuki, 1929) that the heart pulsation of *O. circumpicta* P. from the waters of the northern part of Japan (Anomori Prefecture) is about 14 times per minute at 20° C. In contrast, the pulse of *O. dendata* Kuster from the bay of Palau, South Sea Islands, where the temperature ranges from 28° to 29° C. throughout the year, was only eight times per minute, and the maximum rate of 22 times per minute was observed in the laboratory at 45° C. The pulsation in the northern species at temperature of 28° to 29° C. was 24 times per minute, and the critical temperature was 35° C. These observations may indicate differences in thermic adjustments of oysters inhabiting cold and warm waters. No general conclusions can be drawn at present from Takatsuki's observations because other factors such as degree of sexual maturity and general conditions of the oyster, which were not reported, may affect the heart beat.

Visual observations can be carried on for short periods of time only, and their usefulness is, therefore, rather limited although their distinct advantage is that the heart is not affected by experimental manipulations. The pulse curve of the heart beating inside the intact pericardium may be obtained by the sphygmograph tambour technique. Continuous recording may be made for several hours before the heart is fatigued by the weight of the writing lever pressing on the pericardium wall and the rhythm and amplitude decrease.

The wave-line curve shown in figure 226 represents the changes in the hydrostatic pressure inside the pericardium, the increase in pressure corresponding to systolic contraction of the ventricle which is followed by the falling of pressure during the diastole when the auricles expand and are gradually filled with blood. The method is not sensitive enough to record separately the contractions of the auricles, which beat shortly before the contraction of the ventricle. In the experiment shown in figure 226 the oyster was kept in about 3 l. of sea water at 22.5° C.; its pulse rate was 18 to 20 times per minute.

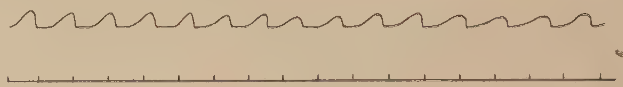

FIGURE 226.—Pulse of an adult *C. virginica* at 22.5° C. recorded by transmitting the motion of the pericardium membrane to the writing lever. Time interval: 3 seconds.

The contractions of auricles interposed between the two ventricular contractions are clearly seen on the tracings of the beats of an exposed heart with the hook connecting the writing lever placed under the auriculo-ventricular junction (fig. 227, two lower lines). In the upper line, the hook was under the ventricle near the emergence of the aorta and the auricular contractions were not registered. The increase in frequency of beat shown in the third (lowest) curve was due to an increase in the water temperature from 20.5° to 24.5° C.

Tracings obtained with the excised heart are similar to those made by the heart in situ with the hook under the ventricle since no contraction of the auricles can be registered in such preparations (fig. 228).

EXTRACARDIAC REGULATION

Carlson (1905a, 1905b, 1905c, 1906a, 1906b, 1906c, 1906d, 1907) has shown that stimulation of the visceral ganglion of *Cardium*, *Pecten*, *Mytilus*, and other bivalves produces an inhibitory effect on the heart. Using faradic stimulation, Diederichs (1935) demonstrated that a single shock applied to the visceral ganglion of *Mytilus* produces diastolic arrest. By separating the ganglia he obtained evidence that both the ac-

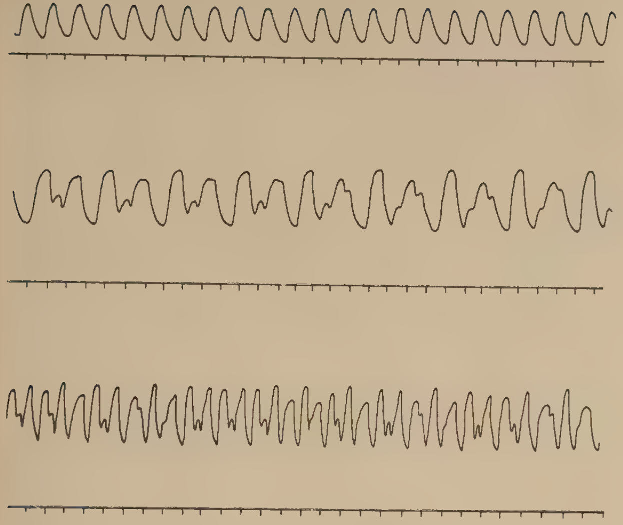

FIGURE 227.—Three records of heart beat of *C. virginica* in situ. The upper curve was obtained by placing the connecting hook of a kymograph lever under the ventricle. The two lower curves were made when the hook was placed under the auriculo-ventricular junction. Increased frequency of the lowest curve is associated with an increase of temperature of sea water from 20.5° to 24.5° C. Time interval, 2 seconds.

celerating and inhibiting nerves lead from the visceral ganglion to the heart and that the two other ganglia affect the heart by way of the visceral ganglion. Oka (1932) found that stimulation of the visceral ganglion inhibits both auricular and ventricular rhythms, and Irisawa, Kobayashi, and Matsubayashi (1961) determined the action potentials in *O. laperousi* and found that oyster heart relaxes through anodal current.

The cardiac nerve is a small branch of the visceral nerve which emerges from the cerebrovisceral connective near the visceral ganglion. Its branches enter the auricles at their base and regulate only the auricular rhythm. The ventricular rhythm, according to Oka's view, is regulated by the cardiac nerves which enter the ventricle at the aorta end. This finding is not in agreement with Carlson's observations that the cardiac nerves enter the heart of a bivalve at the base of the auricles and not at the aortic end. Experimentation with the oyster heart is difficult because exposure of the ganglion causes profuse bleeding and collapse of the heart. Furthermore, the cardiac nerves in *C. virginica* are extremely small and difficult to observe in the living tissue.

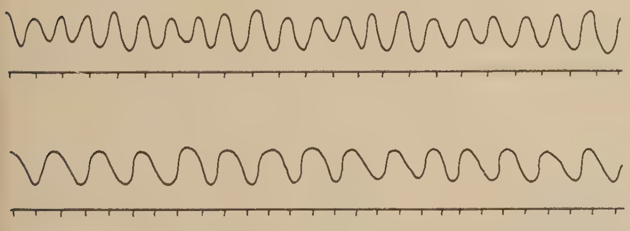

FIGURE 228.—Tracings of the beating of the excised heart of *C. virginica* at 20° C. Salinity 31.7 °/₀₀. Time interval, 5 seconds.

Investigations by Carlson did not demonstrate the presence of acceleratory nerves in the hearts of bivalves. Oka (1932) thinks that possibly both kinds of nerves, the acceleratory and the inhibitory. are present in the heart of *O. circumpicta* but that the action of the inhibitory nerve predominates. The suggestion is based on the observation of old heart preparations of lowered vitality in which the beat of the auricles was slightly accelerated by stimulation of the ganglion. The evidence is not convincing and requires verification.

Krigsman and Divaris (1955) arrive at the following conclusions which appear to be applicable to the oyster heart: 1) The systolic mechanism is situated in the heart's muscle fibers; and 2) extrinsic regulatory nerves influence the pacemaker system. The inhibiting fibers are probably cholinergic, and the accelerating fibers may have adrenergic properties. The latter statement needs further verification.

EFFECTS OF MINERAL SALTS AND DRUGS

Bivalve hearts respond readily to changes in the chemical composition of water and to the presence of low concentrations of various drugs and poisons. Because of this sensitivity the hearts of several common species such as *Anodonta*, *Mya*, *Mercenaria*, *Ostrea*, and others often have been used in pharmacological bioassays. The test is usually made with a preparation of an excised entire heart (or ventricle) in the perfused chamber. Increased acidity slows the beat of the excised heart of *C. virginica;* a pH of 4.0 and lower causes diastolic arrest and from pH 4 to 9 the rate increases with the increase of pH values. Above pH 9 the contractions become irregular (Otis, 1942).

A change in the balance of metallic ions in the surrounding water affects cardiac activity. Small excesses of potassium stimulate the heart by increasing the frequency of beat (positive chronotropic effect) and by changing the tonus (tonotropic effect) of the myocardium (Jullien and Morin, 1930, 1931b).

The action of sodium is similar to that of the potassium, but response is less pronounced. Small excesses of calcium cause negative chronotropic and positive tonotropic effects, and magnesium acts in a way similar to that of calcium, i.e., produces negative chronotropic effect and causes diastolic arrest of the heart. Lack of magnesium results in a systolic arrest (Jullien and Morin, 1931b; Jullien, 1936a).

Among the effects of various drugs the most interesting is that of acetylcholine, a chemical agent in neuromuscular transmission which depresses heart action of oysters and other mollusks (Jullien, 1935c; Jullien and Vincent, 1938; Jullien, Vincent, Vuillet, and Bouchet, 1939; Prosser and Prosser, 1938; Prosser, 1940, 1942; and Wait, 1943) and is particularly effective on the heart of the clam (*Mercenaria mercenaria*). Prosser (1940) has shown that inhibition of the heart of this species can be obtained with a concentration as low as 10^{-12}. Recent investigations by Pilgrim (1954) and Greenberg and Windsor (1962) showed that in the hearts of many bivalves acetylcholine produces a "combination response", depressing the cardiac activity in low concentrations and exciting it at high concentrations. The authors used ventricle strip preparations of the hearts of 40 American (in the Greenberg and Windsor experiments) and 8 New Zealand species (in Pilgrim's tests). Preparations which remained quiescent when first set up attained regular rhythm in 2 to 3 hours, a condition which was also observed in tests made in the Woods Hole laboratory on *C. virginica*. In Greenberg's and Windsor's experiments the quiescent preparations were induced to beat with 10^{-7} to 10^{-5} molar concentrations of 5-hydroxytryptamine.

There exists great variability in the responses of different bivalve species to acetylcholine. In some of them only the depressing effect of the drug was recorded. This group includes oysters (*C. virginica* and *C. gigas*), several clams of the family Veneridae (*Mercenaria mercenaria*, *Tapes philipinarum*, *Saxidomus giganteus*, and others), *Mya arenaria*, *Entoderma saxicola*, and *Prododesmus macroschisma*. The excitor effect was demonstrated for *Mytilus californianus* and *M. canaliculus* (in Pilgrim's tests), thus confirming previous observations on Mytilidae by Jullien and Vincent (1938). In Pectinidae, Matridae, Carditidae, and other families, both types of responses were recorded.

The following explanation of the "combination response", i.e., depression in low concentration and excitation in high concentration, was suggested by Pilgrim (1954): the low concentration tends to inhibit pacemaker activity; at high concentration, while the pacemaker is inhibited, the drug acts directly on the muscle causing a steady contraction. Further research is needed to corroborate this hypothesis.

Greenberg and Windsor (1962) remark that "a reasonable mode of acetylcholine action on bivalve hearts should involve either two separate sites of action or two modes of attachment to the same site at high and low concentrations".

Sensitivity of bivalve hearts to acetylcholine varies in different species. The most sensitive ones, reported by Pilgrim, are *Dosinia*, *Amphiderma*, and *Mercenaria mercenaria*. Oysters are less responsive to the drug. Jullien (1935c) reported that in *C. angulata* the frequency and the amplitude of heart beat are decreased in a concentration of 10^{-5} with diastolic arrest following at two times 10^{-5} to two times 10^{-4} concentration. In New Zealand species, *Ostrea hefferdi*, the cardiac activity is depressed with a diastolic arrest at concentrations varying from 10^{-8} to 10^{-5} (Pilgrim, 1954). In *C. virginica* the decrease in the frequency and amplitude of isolated heart was apparent at concentration 10^{-5} (fig. 229) and the effect persisted for several minutes after the preparation was flushed with fresh sea water (second line). The effect of the drug can be noticed even in extremely low concentrations of 10^{-8} and 10^{-9}. Under normal conditions the hearts of bivalves contain little acetylcholine (Jullien and Vincent, 1938), but the heart of the gastropod *Murex* is very rich in this compound.

Eserine causes periodical alterations in the amplitude of heart beat and slight increase in the rate of beating (fig. 230). The significance of the

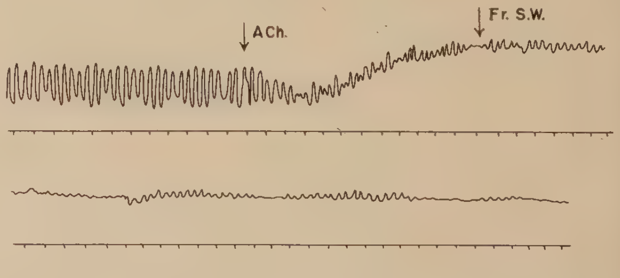

FIGURE 229.—Effect of acetylcholine in the concentration 10^{-5} on the beat of isolated heart of *C. virginica*. ACh—acetylcholine added; Fr.S.W.—perfusion chamber flushed with fresh sea water. Temperature 23.7° C. Time interval, 5 seconds.

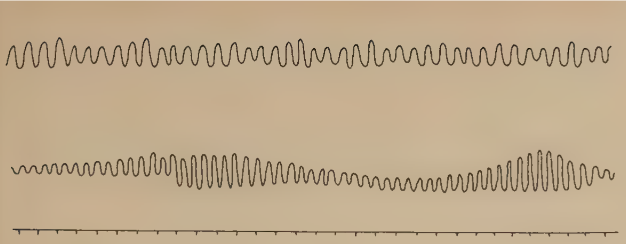

FIGURE 230.—Tracings of the heart beats (in situ) of *C. virginica* in sea water (upper line) and after the addition of eserine, (second line) in concentration of 10^{-4}, to the pericardial chamber. Temperature 21.5° C. Time interval, 5 seconds.

drug in heart physiology is the fact that it prevents the destruction of acetylcholine by the enzymes of the organism.

Veratrine has a temporary stimulating effect on the heart of *O. edulis* (Jullien, 1936a). In my experiments with isolated heart of *C. virginica*, a slight stimulating effect on the frequency of ventricular contraction was recorded in the concentration of veratrine of 1:10,000. Within a few seconds the number of beats increased from 12 to 18 and 20 times per minute at 20.5° C. (fig. 231). Navez (1936) described the depressive action of pilocarpine on the heart of *Anomia*.

High concentrations of curare inhibit the heart activity of the oyster; in lower concentrations the drug has a strong positive tonotropic effect (Jullien, 1936a) and also counteracts the inhibitory effect of acetylcholine. Jullien found that heart action stopped by acetylcholine was restored by subsequent applications of curare.

Adrenaline accelerates the heart beat of *O. circumpicta*, (Takatsuki, 1933) in a concentration of about 1.8 times 10^{-7}. Similar activating action has been reported for *C. virginica* (Otis, 1942) and for *O. edulis* (Jullien, 1935d, 1936a, 1936c). Stronger concentrations produce irregular beating and some times systolic arrest.

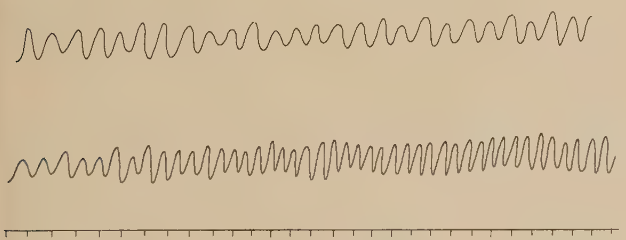

FIGURE 231.—Effect of veratrine (conc. 1:10,000) on ventricular contractions of the isolated heart of *C. virginica*. Temperature 20.5° C. Time interval, 5 sec. Upper line—in sea water; lower line—immediately after the perfusion with veratrine in sea water.

BLOOD VESSELS

Lack of continuity between the arteries and veins due to the presence of sinuses is the characteristic feature of the open circulatory system of bivalves. The spaces which function as capillaries have no distinct walls, are of irregular shape, and appear as slits in the tissue (fig. 79). Their presence imposes difficulty in the maintainance of effective circulation of blood through the organs and tissues. The deficiency is partially overcome by the presence of pulsating vessels and accessory hearts, which assist in the moving of blood through the mantle.

All blood vessels of the oyster have very thin and delicate walls that are easily ruptured by a slight increase in pressure. In anatomical preparations of the circulatory system, it is, therefore, difficult to obtain complete penetration of arterial and venous systems by injection. Partial success may be obtained by using a warm gelatine solution stained with appropriate dyes; by injecting borax or lithium carmine and immediately placing the preparation into 95 percent alcohol in which the stain is precipitated; or by injecting vinyl resin solution diluted with acetone (Eble, 1958). For more detailed study the preparation may be dehydrated and clarified in oil of cloves or in cedarwood oil. Very small vessels may be injected through a capillary tubing using aqueous solution of methylene blue, toluidin blue, or some other suitable dye. Although no permanent preparation can be obtained in this way, the method is useful for tracing the connection between the small vessels.

Because the injection of the venous system is even more difficult than that of the arteries, knowledge of venous circulation in bivalves is less complete than that of the arterial system. Attempts to observe the movement of blood inside the veins usually are not successful because the tissues are either too contractible or contain so much glycogen that the vessels are obscured. The description of the principal blood vessels of the oyster given below is based on the examination of many specimens injected by various methods and studied under a low power of magnification.

THE ARTERIAL SYSTEM

The arteries can be recognized in microscopic preparations by their well-developed walls lined with a single layer of flattened endothelial cells (fig. 81). They have a distinct layer of circular and longitudinal muscles surrounded by connective tissue.

The arterial system described here is shown diagrammatically in fig. 232 from the right side, after the partial removal of the mantle and some of the visceral mass. The right wall of the pericardium is cut off to expose the heart. This diagrammatic drawing is based on examination of several specimens injected through the ventricle.

Two large arteries emerge from the posterio-dorsal side of the ventricle. The largest one is the anterior aorta (ant.ao.), which upon leaving the heart forms a short enlargement or a bulb leading to the large visceral artery (visc.a.) with its numerous branches and small pericardial artery (small unmarked vessel under the visceral artery), which supplies blood to the wall of the pericardium. The much smaller posterior aorta (post.ao.) supplies blood to the adductor muscle and rectum (r.). Near the point of emergence of the posterior aorta it gives off a small rectal artery (r.a.), which follows the wall of the rectum.

The visceral artery (visc.a.) emerges from the anterior aorta as a wide vessel that supplies blood to the organs of the visceral mass. Its upper branch reaches the level of the labial palps and of the cephalic hood. The lower branch extends along the wall of the crystalline sac and forms the reno-gonadial artery (r.g.a.); numerous small branches of this vessel supply blood to gonads and kidneys.

In its course toward the anterior part of the body, the anterior aorta (ant.ao.) passes under the intestinal loop (not shown in fig. 232) and gives off several small vessels which bring blood to the digestive diverticula (gastric arteries, g.a.), mantle, and the labial palps. At the anterior end of the body the aorta forms a common trunk of the pallial artery (co.p.a.), which divides into two short branches corresponding to the left and right side of the body, each branch giving rise to the ventral and dorsal circumpallial arteries (cr.p.a.). Each of these continues along the periphery of the mantle lobes, supplying blood to the mantle through a large number of short vessels which end in the mantle lacunae. A very small subligamental artery emerges from the end of the common pallial artery and leads to the subligamental gland (fig. 78). The cephalic artery (cph.a.) and labial artery (l.a.) supply blood to the anterior end of the body and to the right and left labial palps.

THE VENOUS SYSTEM

Since the presence of irregular sinuses prevents the filling up of the entire venous system with one injection it is necessary to make separate injections of the principal vessels and to supplement the study with an examination of sectioned material. The course of small veins may be traced by injecting a water soluble dye and watching its penetration in the tissues of the visceral mass and gills.

The venous system comprises the sinuses, afferent and efferent veins and small vessels of the gills. It is diagrammatically shown in figure 233. Ramifications of the vessels are omitted for the sake of clarity.

The sinuses occur throughout the entire visceral mass, in the pallium, along the adductor muscle, and around the kidneys. Their outlines are highly irregular, and the area they occupy varies, depending on the degree of distension by blood. The renal sinus (r.s.) consists of several smaller sinuses which surround the main part of the kidneys and open into the efferent branchial vessel and into the sinuses between the adductor muscle and the heart at the posterior side of the body. The renal sinus spreads into the connective tissue of the adjacent area and is in communication with the inter-nephridial passages leading to the pericardium. The renal vein (r.v.) carries blood from the sinus into the common afferent vein. The visceral sinus, v.s., not definitely outlined in the diagram, spreads over the surrounding tissues and drains its blood through the gastric (g.v.), hepatic (h.v.), and other veins into the common afferent vein (c.af.v.). The muscle sinus (m.s.) is a small area below the renal sinus on the surface of the adductor muscle under the pyloric region. The system of afferent veins consists of a single common afferent vein (c.af.v.) and two lateral afferent veins, l. af. v. (fig. 233 and fig. 73). The common afferent vein runs on the ridge formed by the fusion of the two inner ascending lamellae of the gills. The blood received by this vein comes from the deeper parts of the body and is brought by a number of veins which can be identified as the cephalic veins (c.v.) from the cephalic region; the labial veins (l.v.); the gastric and hepatic veins (g.v., h.v.); the network of small reno-gonadial veins (r.g.v.); short renal vein (r.v.) and the adductor muscle vein (not shown in the diagram). In thin, watery specimens most of these veins can

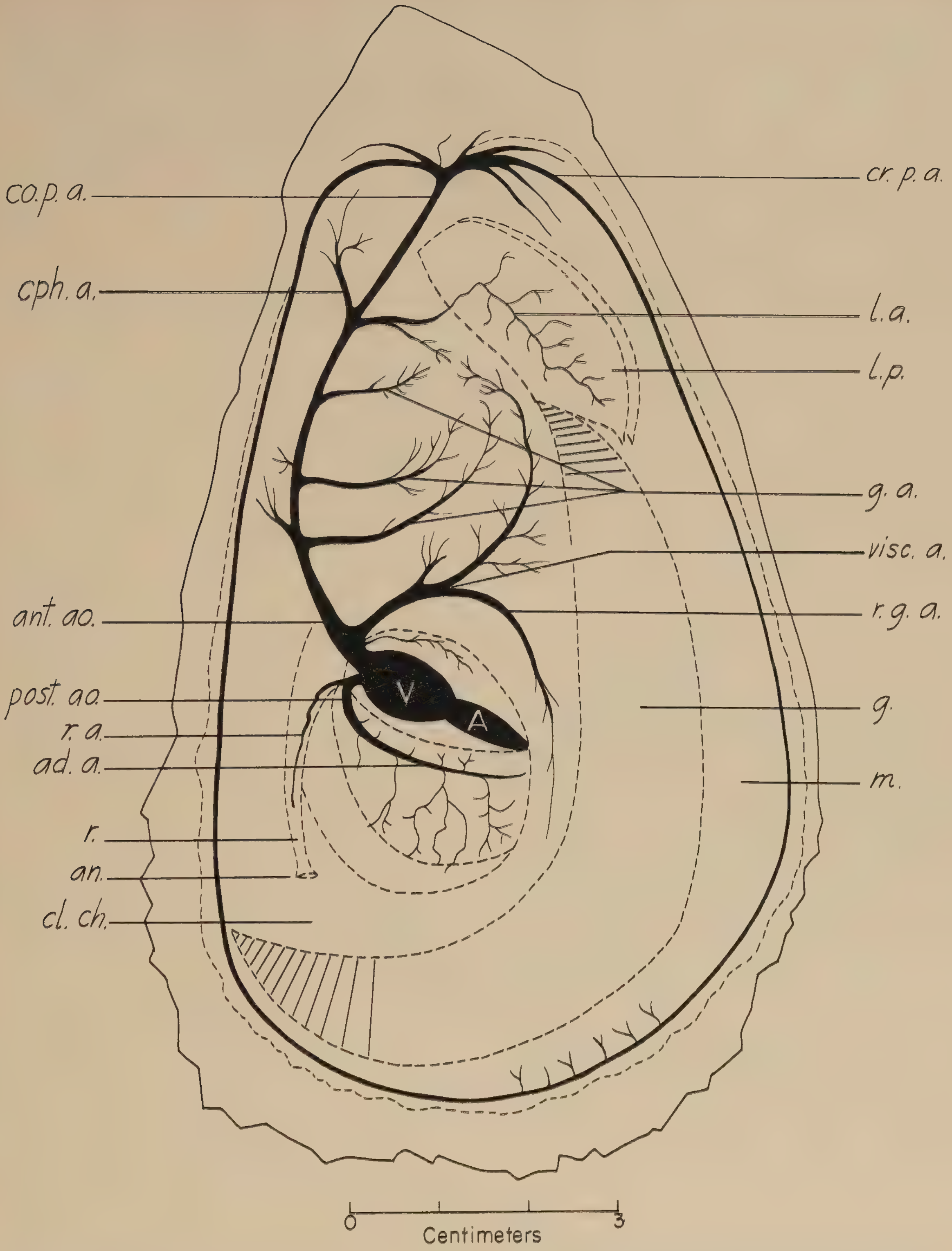
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FIGURE 232.—Diagram of the arterial system of *C. virginica.* A—right auricle; ad.a.—adductor muscle artery; an.—anus; ant.ao.—anterior aorta; cl.ch.—cloacal chamber; co.p.a.—common pallial artery; cph.a.—cephalic artery; cr.p.a.—circumpallial artery; g.—gills; g.a.—gastric arteries; l.a.—labial palp artery; l.p.—labial palps; m.—mantle; post.ao.—posterior aorta; r.—rectum; r.a.—rectal artery; r.g.a.—reno-gonadial artery; visc.a.—visceral artery. For the sake of clarity profuse ramifications of the vessels are not shown.

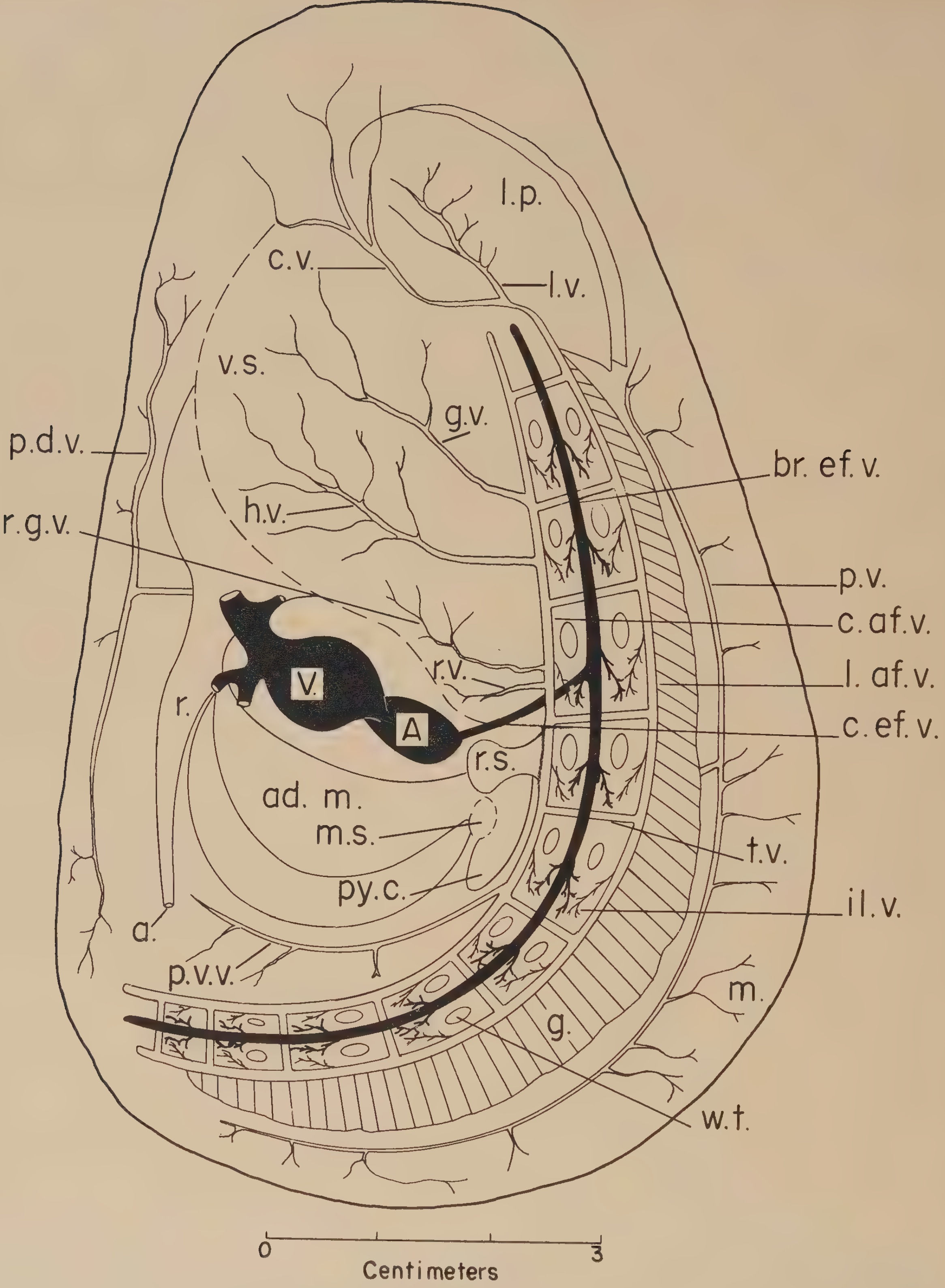
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FIGURE 233.—Diagram of the venous system of *C. virginica* viewed from the right side. The right demibranch is open and pulled out to show the water tubes and the vessels of the descending and ascending lamellae. The left demibranch is not visible. Vessels carrying oxygenated blood are shown in solid black; others are open. The diagram

be seen from the surface. In sexually mature and "fat" oysters they are obscured by the deposits of glycogen and by the accumulation of sex cells. The paired lateral afferent veins (l.af.v.) are of smaller diameter than their common partner. They are located along the axis of the outer ascending lamella where the lamella fuses with the mantle lobe. The lateral afferent veins receive the blood from the mantle through the pallial veins (p.v.).

At regular intervals the common afferent vein is connected with the lateral veins by short transverse (horizontal) vessels (t.v.). These vessels can be seen in injected preparations of the gills and in sectioned material. The communication between the horizontal vessels in the gill tissues is maintained by means of vertical vessels which emerge from the walls of the three afferent veins as a series in a double row, one following the inner and the other the outer lamella of the demibranch. At each interfilamentar shelf the vertical vessels empty into a lacuna and eventually into the tubes of the gill filaments. There is no special path for the return of the blood from the interfilamentar lamellae and the tubes because the filaments end blindly. The walls of the common afferent vein contain a layer of elastic fibers arranged circularly; they are scarce in the walls of other veins. Endothelium is absent in all these vessels. The walls of the vertical vessels of the lamellae have a layer of muscular fibers which are able at intervals to constrict the lumen of the vessels along their length. In this way the flow of blood inside the gills is regulated (Elsey, 1935).

The blood channels in the interlamellar junctions are in communication with the vertical vessels and provide for the passage of blood from one lamella to the other. This rather inefficient circulation of the blood in the gill vessels is influenced by the contraction of the entire gill musculature and by contractions of the major afferent and efferent veins. The pulsations of these vessels have not been observed in vivo, but their histological structure suggests that they are capable of constricting their lumen. A tangential section of the common afferent vein preserved in a relaxed state (fig. 234) shows a well-developed layer of circular muscles flanked on both sides by thin bands of longitudinal muscles.

The system of efferent vessels comprises two short common efferent veins (c.ef.v.) which open into the auricles, a pair of branchial efferent veins (br.ef.v.) which run along the axis of the gill lamellae (fig. 73), pallial efferent veins (not shown in fig. 233), and the interlamellar and interfilamental vessels (il.v.) of the gills. The branchial efferent veins (br.ef.v.) run along the gill axis parallel to the branchial nerves (fig. 73) at the junctions of the ascending and descending lamellae. In their course they receive blood from the renal sinuses and empty into the common efferent vein. Blood which circulates in the mantle is carried to the heart through pallial sinuses and veins, but part of the blood from the posterior portion is drained back to the gills and to the branchial efferent vein (br.ef.v.).

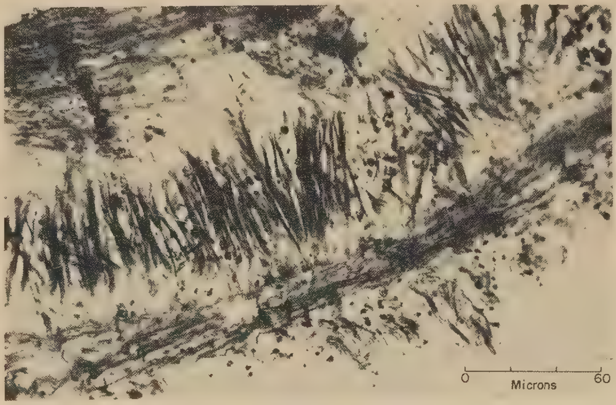

FIGURE 234.—Photomicrograph of a tangential section of the wall of the common afferent vein of *C. virginica* preserved in fully relaxed state. Narcotized in magnesium sulfate. Kahle, hematoxylin-eosin.

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was drawn from a number of preparations of partially injected venous system. Only the approximate position of various vessels is indicated. The diagram does not intend to show the actual appearance and distribution of veins. A—auricle; V—ventricle; a.—anus; ad. m.—adductor muscle; br. ef. v.—branchial efferent vein; c. af. v.—common afferent vein; c. v.—cephalic vein; c. ef. v.—common efferent vein; g.—gills; g. v.—gastric veins; h. v.—hepatic veins; il. v.—interlamellar veins of the gills; l. af. v.—lateral afferent vein; l. p.—labial palps; l. v.—labial vein; m —mantle; m. s.—mantle sinus; p. v.—pallial vein; py. c.—pyloric caecum; p. d. v.—posterior dorsal vein; p. v. v.—posterio-ventral vein; t. v.—transverse veins of the gills; r.—rectum; r. s.—renal sinus; r. g. v.—reno-gonadial veins; r. v.—renal vein; v. s.—visceral mass; w. t.—water tubes of the gills.

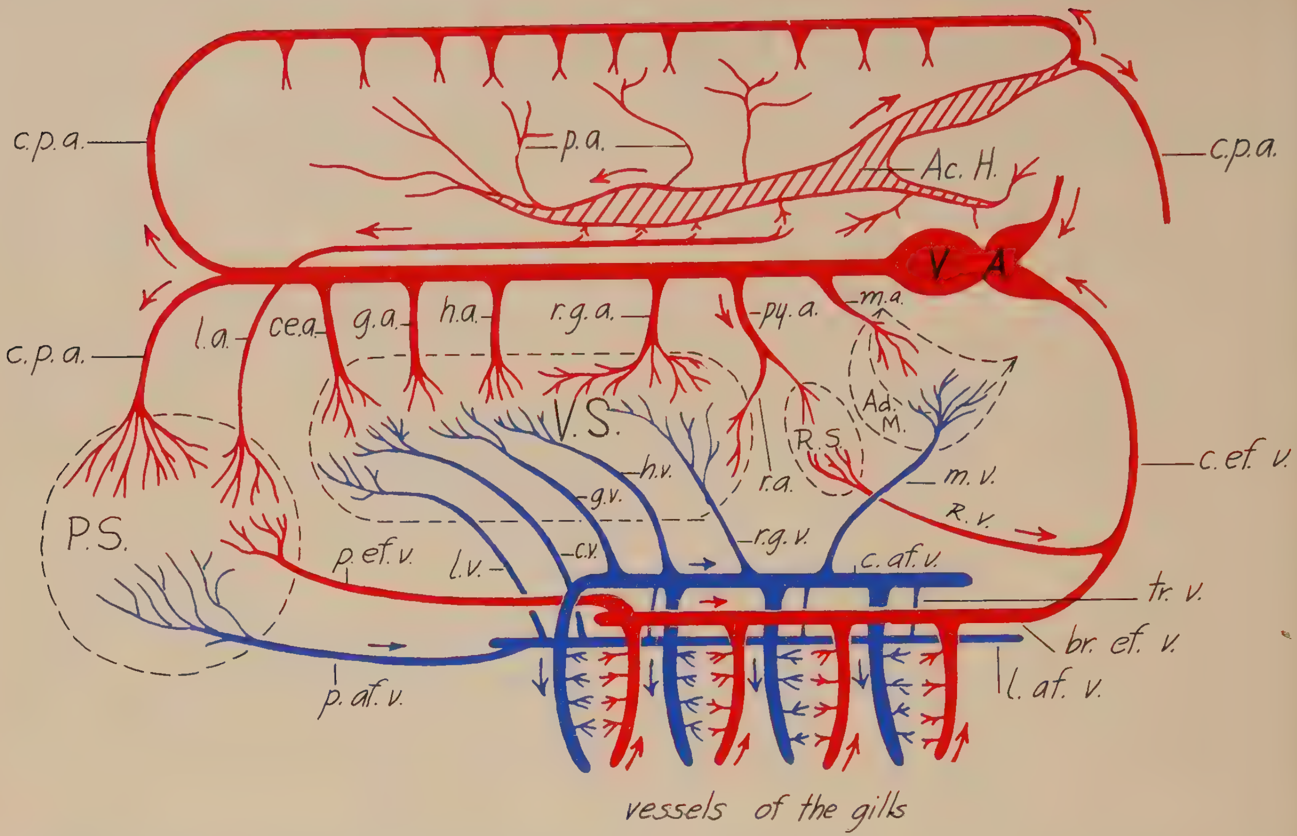

FIGURE 235.—Diagram of the circulation of blood in *C. virginica.* The position of various sinuses marked with capital letters is indicated by broken lines; only one demibranch and one accessory heart are shown. A—auricles; A.cH.—accessory heart of one side; P.S.—pallial sinuses; R.S.—renal sinuses; V—ventricle; V.S.—visceral sinuses; br.ef.v.—branchial efferent vein; c.af.v.—common afferent vein; c.ef.v.—common efferent vein; ce.a.—cephalic artery; cp.a.—circumpallial artery; c.v.—cephalic veins; ga.a.—gastric artery; g.v.—gastric vein; h.a.—hepatic artery; h.v.—hepatic vein; l.a.—labial artery; l.af.v.—lateral afferent vein; l.v.—labial vein; m.a.—adductor muscle artery; m.v.—adductor muscle vein; p.a.—pallial arteries; p.af.v.—pallial afferent vein; p.ef.v.—pallial efferent vein; py.a.—pyloric artery; r.a.—renal artery; r.g.a.—reno-geonadial artery; r.g.v.—reno-gonadial vein; r.v.—renal vein; tr.v.—transverse veins of the gills.

In visualizing the circulation of blood within the gills one must keep in mind the location of the five horizontal vessels at the top of the duplicated W-shaped junctions of the gill lamellae (fig. 73).

The course of circulation presented schematically in fig. 235 shows that the arterial blood goes to the sinuses (P.S., V.S., R.S.) and then is conveyed through the afferent veins to the gills and reaches the auricles via two common efferent veins. Some of the blood from the pallial sinuses (P.S.) and from the renal sinus (R.S.) bypasses the gills and is directly delivered to the auricles through the common efferent veins.

The deficiency in circulation caused by the presence of large sinuses is counteracted by the pulsations of radial vessels of the mantle and by a pair of accessory hearts (Ac.H.), which function independently of the principal heart of the oyster. The red and blue colors of the diagram show that only oxygenated blood fills the heart.

THE ACCESSORY HEART

The accessory heart is a paired tubular structure along the inner surfaces of the right and left mantle folds where they join together to form the cloacal chamber. Its position on the wall of the cloaca and its relation to the adjacent organs are shown in figure 236 drawn from life.

The accessory heart of the oyster is not the simple tubular structure described by Hopkins (1934, 1936) and Elsey (1935). It consists of three branches of almost equal size, joined together at a common center (fig. 237). The entire structure has the shape of the letter Y. The lower or ventral branch (v.br.) extends along the

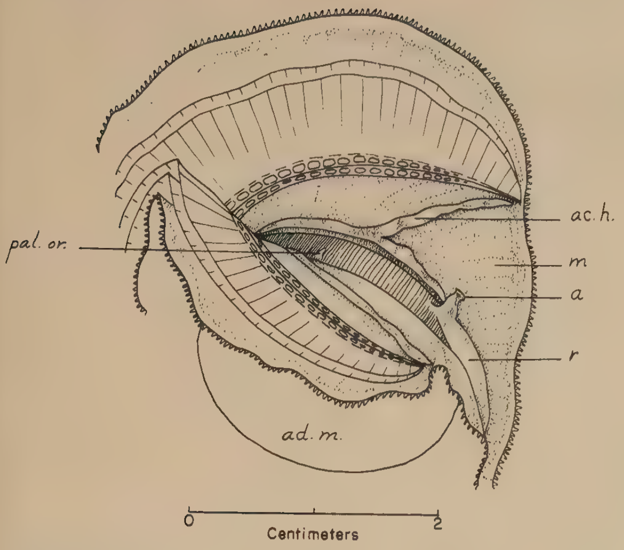

FIGURE 236.—The position of the accessory heart on the left of the cloacal wall of *C. virginica*. The epibranchial chamber was dissected, and the demibranchs of the right and left side pulled apart to expose the ventral side of the adductor muscle. The oyster was fully narcotized. The accessory hearts on both sides were fully expanded (only the part of the right accessory heart is shown). a.—anus; ac.h.—accessory heart on the left side; ad.m.—adductor muscle; m.—mantle; pal.or.—pallial organ; r.—rectum.

wall of the cloaca to the pallio-branchial junction (p.br.j.).

Under slight mechanical stimulation the delicate wall of the accessory heart collapses and the structure becomes invisible. This explains why the earlier investigators did not recognize it as an active organ and mistook it for ridges on the inner wall of the mantle (Rawitz, 1888; Kellogg, 1892).

The structure of the accessory heart of *C. virginica* (fig. 238) resembles that of the arteries of the mantle. The walls have a well-developed layer of longitudinal and circular muscles, but the endothelium lining is indistinct and is probably absent.

The pulsation of accessory hearts of *C. virginica* observed in winter at the Woods Hole laboratory was very irregular, not exceeding two to three times per minute at room temperature of 20° to 22° C., and was independent of the heart beat. During the summer the rate of contraction was six to seven times per minute. Hopkins (1934) states that in *C. gigas* the accessory heart beat at a slower rate than the average heart rythm of this species and the frequencies for right and left organs averaged 6.0 and 7.5 times per minute respectively.

The connection of the accessory heart to other vessels was studied by the following method of injection. Live oysters were kept for 24 to 48 hours in a refrigerator, then placed overnight in cold sea water with 5 percent magnesium sulphate. About 2 ml. of lithium carmine was injected, using the finest hypodermic needle. The preparation was rapidly rinsed in fresh water and immediately immersed in 95 percent ethanol, which precipitated the dye. The injected material remained inside the vessels and was not diffused or washed away by dehydration and clarifying agents (cedar oil or xylene). In this way several permanent preparations were obtained.

Dye injected into the ventral branch (fig. 237, v.br.) penetrated some distance into the circumpallial arteries of the right and left mantle lobes and into the small branches and capillaries of the efferent vein of the gills (ef.v.). The dorsal branch (d.br.) was found to extend along the wall of the cloaca: it does not "disappear into the excretory organs," as stated by Hopkins, but extends under the renal sinus to the dorsal part of the cloacal wall. The ramifications of the branch end in a number of capillaries which connect them with the dorsal portion of the efferent vein. The third or posterior branch (p.br.) follows the ventro-lateral border of the adductor muscle and gives many ramifications inside the cloacal wall.

Blood carried by the ventral branch of the accessory heart enters the pallial artery against the pressure produced by the principal heart. Under these conditions its penetration inside the artery must be limited, and at the end of the contraction wave some of the blood probably re-enters the branch. Movement of the blood inside the circumpallial artery can not be seen, but through the thin wall of the accessory heart one can observe the flushing of blood cells back and forth. Ramifications of the ventral and dorsal branches form capillaries which are in direct connection with the side vessels of the efferent vein. It can be assumed from the direction of the contraction waves that blood from the accessory heart moves toward the efferent vein of the gill and that part of the blood is flushed back as the impulse wave progresses along the wall of the branch.

Oscillation of the blood in the mantle is the primary function of the accessory hearts. Their oscillatory movements facilitate the gaseous exchange and provide a means for efficient respiration. The location of the accessory hearts con-

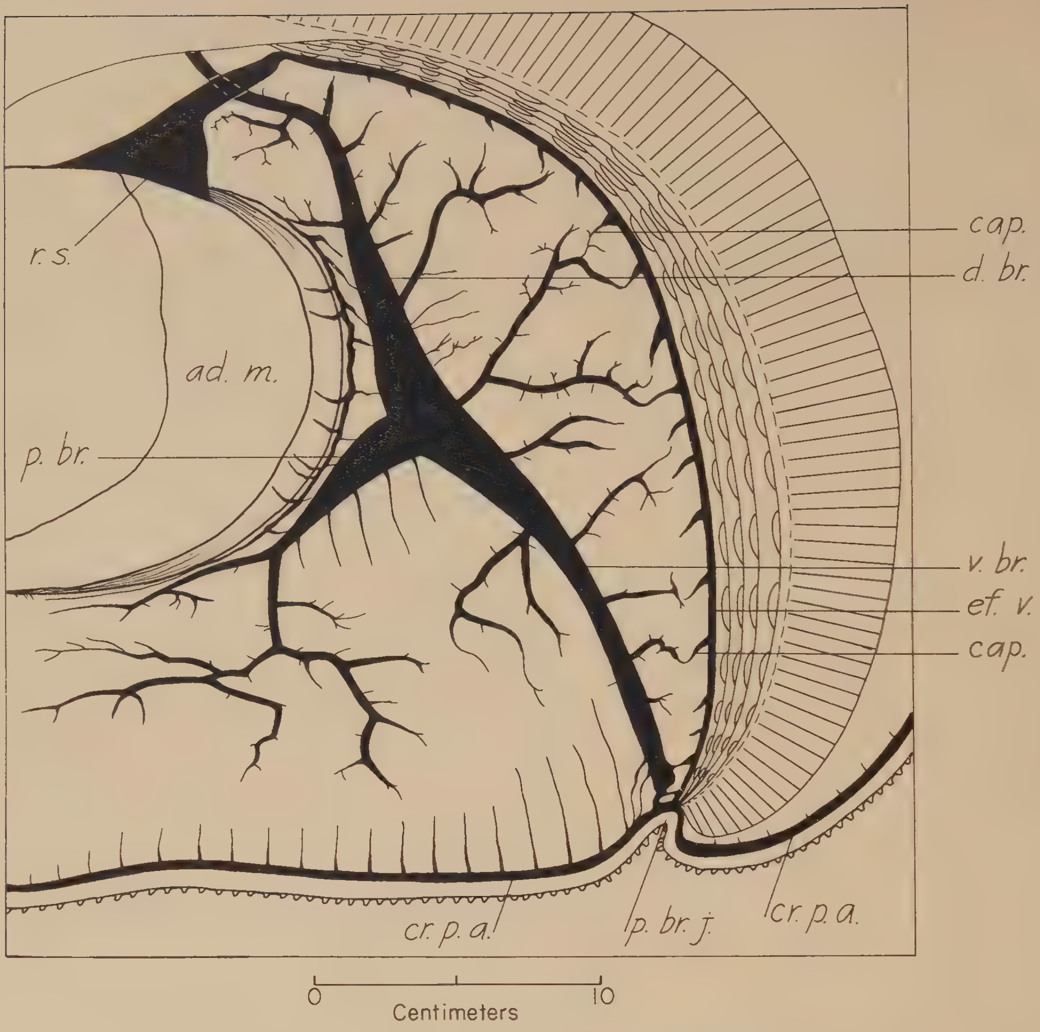

Figure 237.—Accessory heart of *C. virginica*. Drawing made from an injected preparation. ad.m.—adductor muscle; cap.—capillaries; cr.p.a.—circumpallial artery; d.br.—dorsal branch; ef.v.—efferent vein; p.br.—posterior branch; p.br.j.—pallio-branchial junction; r.s.—renal sinus; v.br.—ventral branch.

firms the opinion that the mantle and the wall of the cloaca play significant roles in the respiration of oysters.

The pulsation of the accessory hearts makes it possible for the blood of the pallial sinuses to enter the branchial efferent veins or to be forced into the gills through the lateral afferent veins. The pacemaker system and the nervous control of the accessory hearts have not yet been studied.

THE BLOOD

There are two distinct groups of blood corpuscles in bivalve mollusks, the hyaline cells and the granular amoeboid cells. The latter are frequently called granulocytes because of the large number of granules in their cytoplasms, or amoebocytes and phagocytes because of their ability for amoeboid movements and phagocytosis. The hyaline cells are not entirely devoid of granules but they are very sparse. These cells also display amoeboid movement but are much less active than the granular cells. Both types of cells are present in the oyster.

Samples of blood for examination may be obtained by puncturing the pericardial wall with a fine glass pipette and drawing the desired volume of blood. In the same manner blood may be obtained directly from the ventricle or auricles. Some blood cells are always present in the shell liquor and on the surfaces of the gills and mantle. A fair sample of cells can be obtained by scraping these tissues with cover slips or by drawing the pipette along them. For examination of live

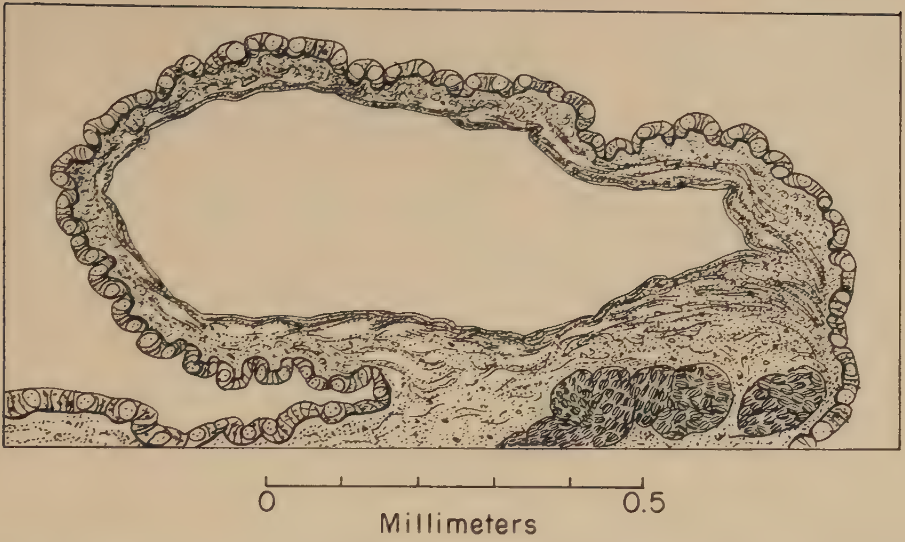

FIGURE 238.—Transverse section of the accessory heart of *C. virginica* preserved in widely expanded state. Kahle, hematoxylin-eosin.

cells the sample may be placed in a moist chamber or a small quantity of blood may be dropped in a glass dish with sea water of the same salinity from which the oysters were taken. Under these conditions the cells of *C. virginica* may remain alive for about 6 days and can be used for studies or classroom demonstration (Breder and Nigrelli, 1933).

For smear preparations drops of blood should be left on slides until the cells begin to expand. When a desired state of expansion has been attained, the preparation is fixed in Bouin III for a few minutes or in chromic or osmic acid (liquid or fumes). Satisfactory preparations may be obtained by using Romanowsky's, Leishmann's, Giemsa's, and McNeal's tetrachrome stains made in a solution of absolute methyl alcohol. These reagents fix and stain the cells in one operation.

COLOR OF BLOOD

The blood of the oyster is colorless and contains no respiratory pigments such as the hemoglobin in vertebrates or hemocyanin found in snails and cephalopods. In semipopular books on oysters a statement is sometimes found about the presence of hemocyanin in oyster blood. To clarify this question, a composite sample of blood and pericardial fluid was collected from six adult *C. virginica* and submitted for spectrophotometrical analysis, which was kindly performed in George Wald's laboratory at Woods Hole. The following is the report received from Wald:

"The pH (of the sample) was 7.33. The absorption spectrum showed specific absorption in the visible region corresponding to the hemocyanin band at about 570 mμ. Hemocyanin possesses also a very high, sharp absorption peak at about 340 mμ., some 20 to 30 times as intense as the absorption in the visible spectrum. This therefore constitutes a very delicate test for the molecule. This also did not appear in the spectrum though a small band was found at lower wavelengths, at about 327 mμ.

"The 570 and 340 mμ. absorptions are found in oxyhemocyanin; both are abolished in the reduced condition. As an added test therefore this sample of oyster blood was reduced with sodium hydrosulfite. The ultraviolet absorption at about 327 mμ. instead of being depressed, rose greatly. I do not know what this substance is, but quite certainly it is not hemocyanin."

THE HYALINE CELLS

These cells with clear cytoplasm containing but few granules are of uniform shape, varying only in size from 5 to 15 μ. When examined alive they are usually spherical (fig. 239), have a distinct cell membrane, and are of pale yellow-green color. Because of their high refractive quality they stand out sharply in the field of view of the microscope. The slow movement of the cells can be noticed if the preparation is watched intently for 30 minutes or longer. One of these cells, under continuous observation in the Woods Hole laboratory for 45

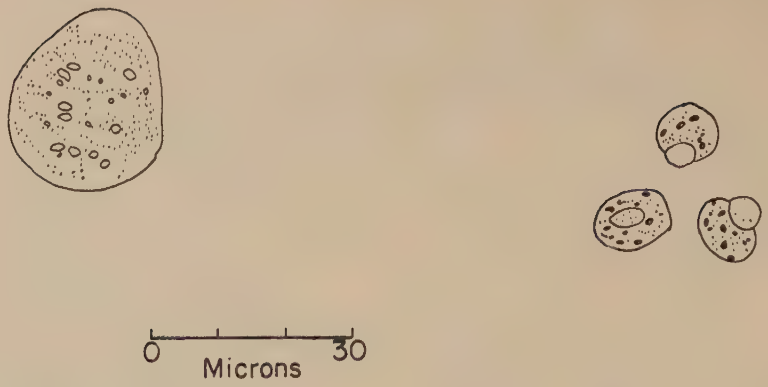

FIGURE 239.—The hyaline blood cells of *C. virginica*. Very small cells on the left; normal cells on the right. Camera lucida drawing of live cells on glass.

minutes changed its shape four times from round to oval and back again. The movement is extremely gradual and consists mainly in bulging of one side of the body. The nucleus is not visible in the live cells and rarely can be seen in stained preparations. The cells are basophilic, staining reddish-purple with Romanowsky's stain. The nucleus stains the same color as the cell.

The hyaline cells comprise about 40 percent of the total number of blood cells in a sample. This is an average of a number of samples taken from the oysters of Long Island Sound and of Chesapeake Bay in which blood was drawn from the pericardium, heart, and shell liquor. In the oysters in good, healthy condition, the proportion of hyaline cells varied from 25 to 64 percent, but the differences were not consistent and did not seem to be affected by the origin of the oysters or by the part of the body from which the sample was taken.

THE GRANULAR CELLS

The granular cells or the amoebocytes vary greatly in shape, size, and behavior. This undoubtedly is due to their pronounced ability for amoeboid movement. In live contracted state they measure about 6 μ in diameter, but they expand and spread to a much larger size. When fresh blood drawn from the oyster by a pipette is spread on a glass slide, many blood cells form aggregates or clumps. This aggregation or agglutination results from the adhesiveness of the cell membranes, which stick on contact with one another (Drew, 1910). In a quiescent stage the cells are usually round and motionless. In about half an hour they begin to expand and separate from the clump. By the end of the first hour the amoeboid movement becomes active and the cells disperse themselves and form concentric rings around the clump.

The cytoplasm and the granules of a moving amoebocyte (fig. 240) flow slowly from the center of the cell out to the edge and push the cell membrane out, forming a pseudopodium. During the formation of very narrow pseudopodia the cytoplasm appears to flow out with the granules arranged in single file. Contraction seems to be affected all at once over an entire cell area, and the action can be quite sudden. In withdrawing, the cytoplasm sometimes leaves behind it a colorless and empty membrane. Fine hyaline projections called "bristle pseudopodia" (fig. 240, right) may remain extended from the membrane and some can be traced back to it. This seems to confirm the argument of Goodrich (1920) that the bristle type pseudopodium is a fold or thickening in the membrane and not a physiologically active part of the cell body.

Clots of blood cells are often observed in injured blood vessels and the connective tissue surrounding small arteries of the mantle, and can be produced by intercardiac injection of tissue extracts. Infiltration of connective tissue by amoebocytes and intravascular blood clots is usually found in watery green oysters from polluted water (fig. 241).

There is no true coagulation of the oyster blood. The coalescence and clot formation of blood cells outside of the body is the result of the entanglement of amoebocytes by the bristlelike pseudopodia or by the strands of hyaline ectoplasm (fig. 242).

The granules of live amoebocytes are usually yellowish-green, with the color much more pronounced in green oysters. The staining affinities of blood cells have been studied by several in-

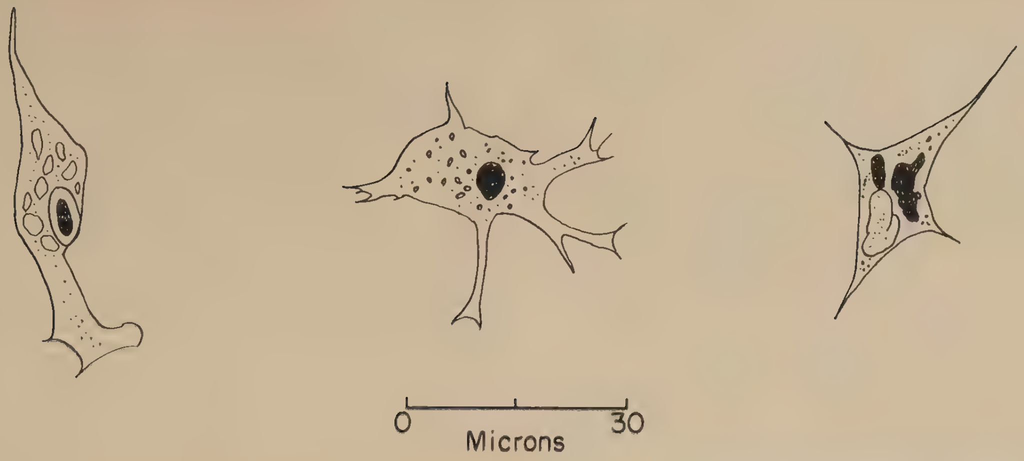

FIGURE 240.—Amoebocytes (granular cells) of *C. virginica* observed in vivo. Camera lucida drawings of live cells on glass.

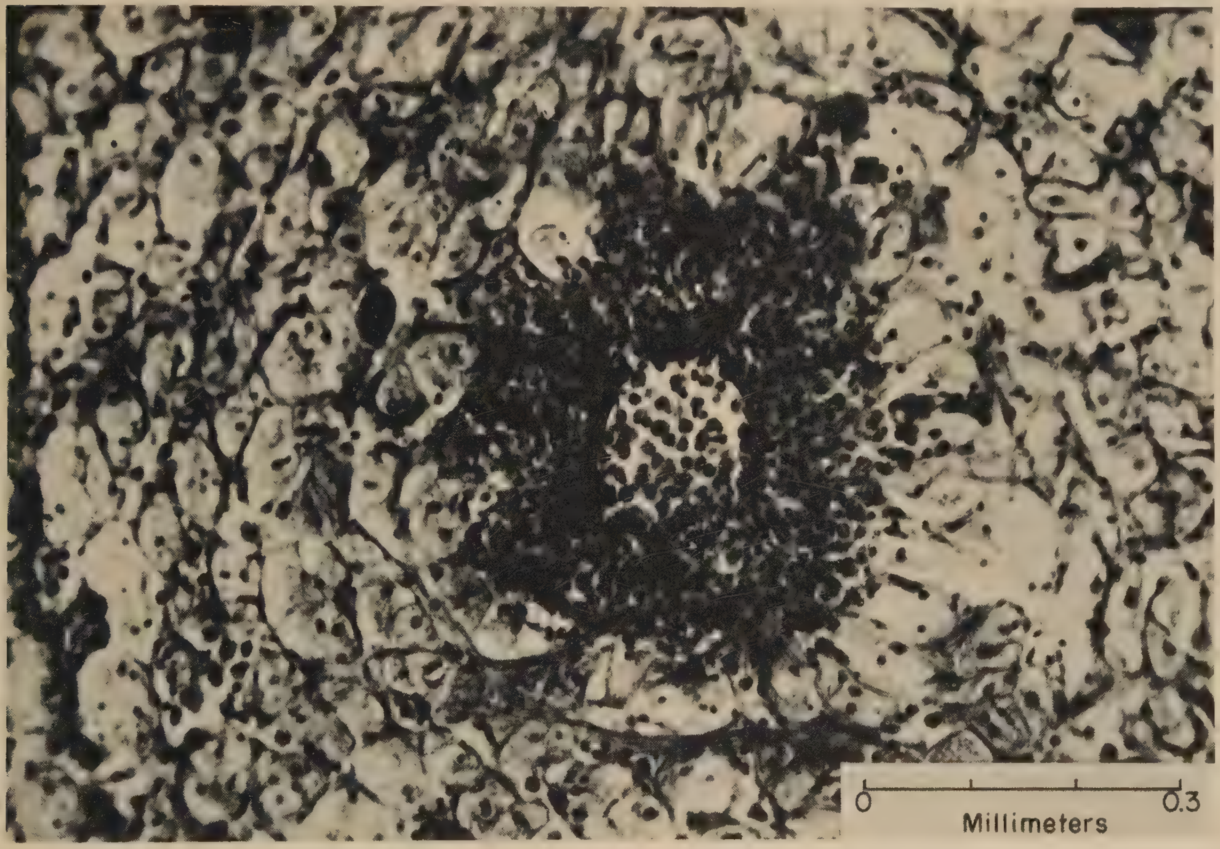

FIGURE 241.—Intervascular blood clot and infiltration of amoebocytes in the mantle of green *C. virginica.* Bouin, hematoxylin-eosin.

vestigators with somewhat different results. Kollmann (1908) found that marine lamellibranchs have acidophilic granules, while those of fresh water mollusks are amphophilic. The granules of *O. edulis* (Takatsuki, 1934a) are neutrophilic with a tendency to become stained vitally by basic dyes. The amoebocytes of *C. circumpicta* (Ohuye, 1938) have eosinophilic or amphophilic cytoplasm and basophilic granules. In a blood smear preparation of *C. virginica* examined in the Bureau's shellfish laboratory the granules stained reddish-purple to dark blue with polychrome

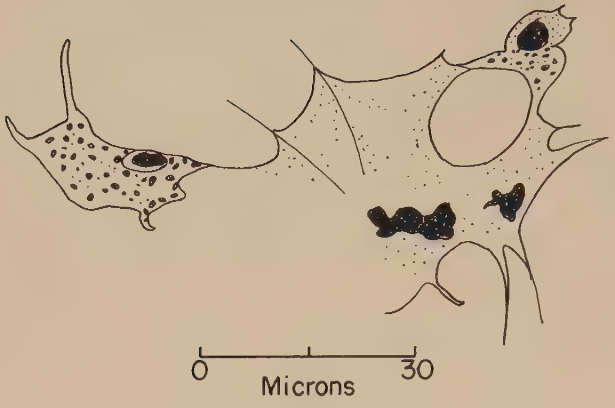

FIGURE 242.—Beginning of coalescence of blood cells of *C. virginica.* Camera lucida drawings of live preparations.

methylene blue mixture (Ramanovsky stain). Methylene blue alone stained the granules very poorly. In Ehrlich triacid stain a few granules were blue, indicating a neutrophilic reaction. In my preparations the blood cell granules never took up eosin, which is very acid stain.

The oval-shaped nuclei of the amoebocytes can be seen easily in a stained preparation. The nucleus is usually located slightly off the center of the cells in a pocket devoid of granules.

Some of the amoebocytes accumulate iron, copper, zinc, and manganese. The presence of heavy metals can be detected by treating the sectioned tissues with ammonium sulfide, which blackens the metals inside the cells (see: chapter XVII).

The following enzymes have been found in extracts of amoebocytes: amylase, glycogenase, lipase, protease, and a complete oxidase system (Yonge, 1926; Takatsuki, 1934a).

Phagocytic activity of amoebocytes is very pronounced. It can be demonstrated by injecting into the mantle or gill cavity various suspensions such as olive oil (stained with Sudan), carborundum, colloidal carbon, carmine, saccharated iron oxide, and cultures of diatoms or *Chlorella.* Some of the suspended particles may be picked up by the amoebocytes which are always present on the surface of the gills and the mantle. Ingestion of iron particles was observed in the Woods Hole laboratory by adding a suspension of iron saccharate to the shell liquor and treating the samples of tissues or smears with ferricyanide solution to produce Prussian blue reaction. Phagocytosis can also be observed in live amoebocytes placed in sea water on glass slides. Frequently under this condition the amoebocyte approaching a bacterium reverses its movement and turns aside. The cause of this failure of phagocytosis has not been determined. According to Bang (1961), who described the phenomenon in *C. virginica,* it was impossible to assign the failure to a particular combination of bacteria and amoebocytes because repeated observations gave inconsistent results. He concluded that there was probably an undiscovered factor in phagocytosis in oyster blood which was responsible for this variation in behavior.

Tripp (1960) found that various species of living bacteria and yeast cell injected in the tissues of *C. virginica* were rapidly destroyed extracellularly and by phagocytes. Bacterial spores were removed from tissues at a much slower rate.

At the beginning of phagocytosis of an uniflagellate bacterium, observed by Bang with the electron microscope (fig. 243), many filamentous pseudopods extend from the cell's surface and entangle the flagellum which is coiled around them while the bacterium remains outside the amoebocyte's body.

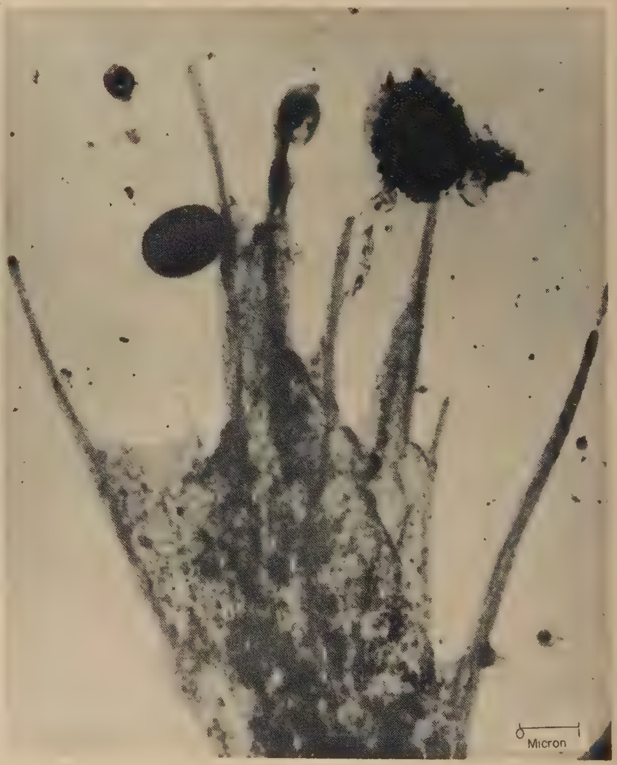

FIGURE 243.—Electron micrograph of a periphery of one amoebocyte which spread out on a collodion film and was fixed with osmium vapor. Courtesy of F. B. Bang.

SPECIFIC GRAVITY OF BLOOD

The osmotic pressure of body fluids of bivalves is about equal to that of the surrounding water so it may be expected that the specific gravity of blood approximates that of the water. For determining the specific gravity of blood or of pericardial fluid, the falling drop method of Barbour and Hamilton (1926) has been used. The procedure consists of timing a drop of fluid of uniform size as it falls a distance of 30 cm. through a mixture of xylene and bromobenzene in a vertical glass tube of exactly 7.5 mm. in diameter. The time is recorded with a stopwatch accurate to one-tenth of a second. The speed of falling of a drop of the sample is compared with that of a drop of the same size of standard potassium sulfate (K_2SO_4) solution of known density. By using an alignment chart (supplied with the instrument), correction is made for room temperature; the specific gravity of the sample can be calculated with an accuracy of 1 times 10^{-4}. The source of error caused by variations in the size of drops is minimized by using an automatic Guthrie pipette controller. The method is simple, rapid, and gives consistent results. In this way the specific gravity of blood was determined for oysters taken from various environments.

A series of tests was also made to record changes that occurred in oysters placed in diluted sea water and in those exposed to air. The blood collected from the ventricle with a glass pipette was centrifuged for 20 minutes at 1,200 r.p.m. to separate blood cells from plasma. For brief storage the sample of plasma was kept in a paraffin coated container from which portions were taken for determination. Observations were made at the time of full sexual maturity of the oysters in the middle of July and were repeated 2 weeks later at the completion of spawning. All tests were made at 22° C. and salinity 31.0–31.5 $^{\circ}/_{\circ\circ}$. The oysters were collected from Wellfleet Harbor, Mass., but remained in the laboratory tanks for about 3 weeks before the tests. The specific gravity of blood during the July 15 to 18 period varied from 1.0252 to 1.0262; in the tests made after spawning between July 28 and 31 the specific gravity of blood varied from 1.0258 to 1.0259. The results are close to those reported by Yazaki (1929) for *O. circumpicta* in which the specific gravity of blood in the summer specimens varied between 1.025 and 1.029.

No significant changes were found in the blood of oysters kept for 72 hours in the refrigerator at temperatures varying from 4.5° to 7.5° C. At the end of the test the specific gravity of the blood of the refrigerated mollusks was 1.0258; and in the controls which were kept in running sea water at 21° to 22° C. the blood was 1.0259.

A gradual decrease in specific gravity occurred in the oysters kept in running sea water of diminishing salinity. The results of this experiment are shown in table 31.

In highly diluted water shell movements of some of the oysters were abnormal and most of the time they remained closed. In these oysters the specific gravity of the blood after 72 hours of exposure to salinity of 9 to 12 $^{\circ}/_{\circ\circ}$ was relatively high (1.0138 and 1.0178) compared to the specific gravity of 1.0092 in the oysters which stayed open for more than 50 percent of the total time. It may be deduced from these experiments that the oysters kept in water in which the salinity was reduced from 31–32 $^{\circ}/_{\circ\circ}$ to 16.7–17.7 $^{\circ}/_{\circ\circ}$ attained the osmotic equilibrium of blood in about 120 hours.

Table 31.—*Decrease in the specific gravity of cell-free blood of the oyster,* C. virginica, *in water of lowered salinity*

Salinity ($^{\circ}/_{\circ\circ}$)	Time			
	24 hours	48 hours	72 hours	120 hours
31–32, control	1.0259	1.0259	1.0259	1.0259
16.7–17.7	1.0145	1.0143	1.0143	1.0127
9–12	1.0199	1.0103	1.0092	(*)

*Observations discontinued after 72 hours.

SEROLOGY

Serological reactions between several mollusks were studied by Makino (1934), who experimented with the following species: bivalves—*Meretrix meretrix, Paphia philipinarum, Ostrea* (*Crassostrea*) *gigas, Arca inflata;* gastropods—*Turbo cernutum, Haliotis gigantea, Rapana thomasiana;* cephalopods— *Sepiella japonica* and *Polypus variabilis.* In these tests the extracts of tissues in physiological saline solution were injected intraperitoneally or subcutaneously into rabbits to obtain the antisera. Injections were repeated for 7 days using doses which increased from 0.5 to 5 grams. One ml. of extract and 0.1 ml. of antiserum were used in performing precipitation tests, and the tube was set aside for 1 hour at 37° C. Positive reaction was obtained with all the species. *Ostrea* antiserum reacted very strongly with *Meretrix* and *Paphia* and less strongly with

Turbo, Haliotis, and *Rapana.* It is interesting to note that *Arca,* which belongs to the phylogenetically low order of Protobranchia, reacted very strongly not only with *Meretrix* and *Ostrea,* but also with the gastropods *Turbo, Haliotis,* and *Rapana.*

Wilhelmi (1944) applied the precipitation reaction to the problem of determining the relationship of the mollusca to other invertebrates. Using a technique similar to that employed by Makino, he made tests between two species of *Busycon, Pecten irradians, Nereis, Limulus,* and *Asterias forbesi* and concluded that, serologically, mollusca are more closely related to annelids than to any other group. At present this work has historical interest only, since it is obvious that no broad speculations about the relationship of various phyla should be made on the basis of a few tests made with only six species belonging to four different phyla.

The existence of serological differences in five bivalves (*Anadara inflata, A. lareta, Pecten yessoensis, Ostrea* (*Crassostrea*) *yessoensis,* and *O. circumpicta*) was demonstrated by Tomita and Koizumi (1951). In this work the serum was obtained by centrifuging the blood withdrawn from the auricles of the mollusk. Antisera were obtained by injecting rabbits with increased doses, starting with 1 ml. and adding 1 ml. each time until 5 ml. were given on the 5th day. Blood was taken on the 9th day after the last injection. In homologous precipitation tests with *C. gigas,* i.e. using the antiserum against the antigen of the same species, positive reaction occurred in 1:16 dilution of antiserum with 1:1280 dilution of antigen.

Finer differences between closely related species were detected by absorption tests. When a cross reaction is obtained in a test of an antiserum of one species against the serum of a related organism, it is assumed that the second organism possesses a chemical substance common with the homologous substances of the first one. If after the absorption the serum still reacts with homologous substance, it is considered that the antiserum contained antibodies to two or more chemical components including the one which is common to both. Using this method Tomita and Koizumi found that absorption with *C. gigas* antigen removed from *C. circumpicta* serum all antibodies for *gigas* but not for *circumpicta.* In another test *circumpicta* removed from *gigas* antiserum all antibodies for *circumpicta* but not for *gigas.* The authors' interpretation is that there are some common antigens between *C. gigas* and *C. circumpicta* but that each also has its own specific antigen. The investigators also found that *Anadara* (*Arca*) has all the antigens possessed by *C. gigas* plus its own specific antigen. This is in accord with the generally accepted view that *Anadara* (*Arca*) is a phylogenetically primitive form. Fresh-water *Anodonta* showed no affinities with any other species tested in this work.

The application of absorption technique enabled Numachi (1962) to show that the four local races of *C. gigas*—Hokkaido, Miyagi, Hiroshima, and Kumamoto—have some antigenic differences that are in accord with their geographic isolation.

Application of serological tests is a very promising method for studies of racial differences among oysters. At present it is not known whether the observed antigenic differences are hereditary characteristics or are caused by differences in local environment and particularly in the diet of oysters from different localities.

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CHAPTER XII

THE EXCRETORY SYSTEM

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End products of bivalve catabolism are excreted by the nephridia, pericardial glands, wandering phagocytes, and the mantle epithelium. The urinary function, which is the principal activity of the excretory system, is performed by the paired nephridia situated on either side of the visceral mass near the heart. The pericardial glands, as the name indicates, are located on the wall of the pericardium but in the oysters and some other species are represented by special cells on the outer wall of the auricles. The wandering phagocytes may be found throughout the tissues of the visceral mass and the gills. They accumulate on the surface of the body by diapedesis and are discarded. Mucus or goblet cells of the surface epithelium are, in addition to their primary function of secreting mucus, involved in excretion and carry within their bodies various granules which contain pigments and heavy metals (see: ch. XVII).

The principal part of the excretory system of bivalves is similar to that of annelids. It consists of a pair of tubular nephridia (fig. 244, 1. neph., r. neph.) which retain direct communication with the pericardium and sex glands on one side and open to the outside through a short passage. The coelom of bivalves is reduced to two separate spaces, the pericardial cavity and the inside of the gonad tubules. The ducts leading from the nephridia and gonads open to the outside either independently or through a common reno-gonadial vestibule.

Comprehensive reviews of the anatomy and histology of the excretory system in mollusks may be found in the papers of Odhner (1912), Strohl (1924), Haas (1935), Spitzer (1937), and Franc (1960).

ANATOMY OF THE EXCRETORY SYSTEM

The excretory organ of the oyster occupies an indistinctly outlined triangular area on either side of the visceral mass. On the surface its location is marked by light brownish pigmentation. The organ consists of a central part which lies between the pericardium and the adductor muscle and two branches or limbs which extend along both sides of the body. The right limb is slightly longer than its opposite member. Excretory tissues are found directly under the surface epithelium; they are surrounded by branchial vessels and numerous blood sinuses.

The relation between the different parts of the excretory system, the heart, and the adductor muscle is shown diagrammatically in figure 244.

Each nephridium of a bivalve is bent into a U-shaped tube which forms many convoluted branches penetrating the surrounding tissues. The narrow central part of the excretory system extends across the body from one side to the other and contains a narrow and twisted inter-nephridial passage (i.r.p.) which connects the two limbs. Fingerman and Fairbanks (1958) think that this passage "does not appear to be present in *Crassostrea virginica*." Reconstruction of the structure of this area from serially sectioned material shows that there is a communication between the right and left nephridia although the passage is narrow and greatly twisted (fig. 245) and, therefore, appears in the preparations as a series of circles and irregular cylinders. Examination of a number of consecutive sections shows an uninterrupted connection between the right and left nephridia.

The nephridium of each side begins inside the pericardium by the reno-pericardial opening (fig. 244, r.p.o.), which leads through a ciliated funnel, the nephrostome and a short reno-pericardial canal (fig. 244, r.p.c., and fig. 246), to the body of the kidney. Both limbs of the kidney are formed

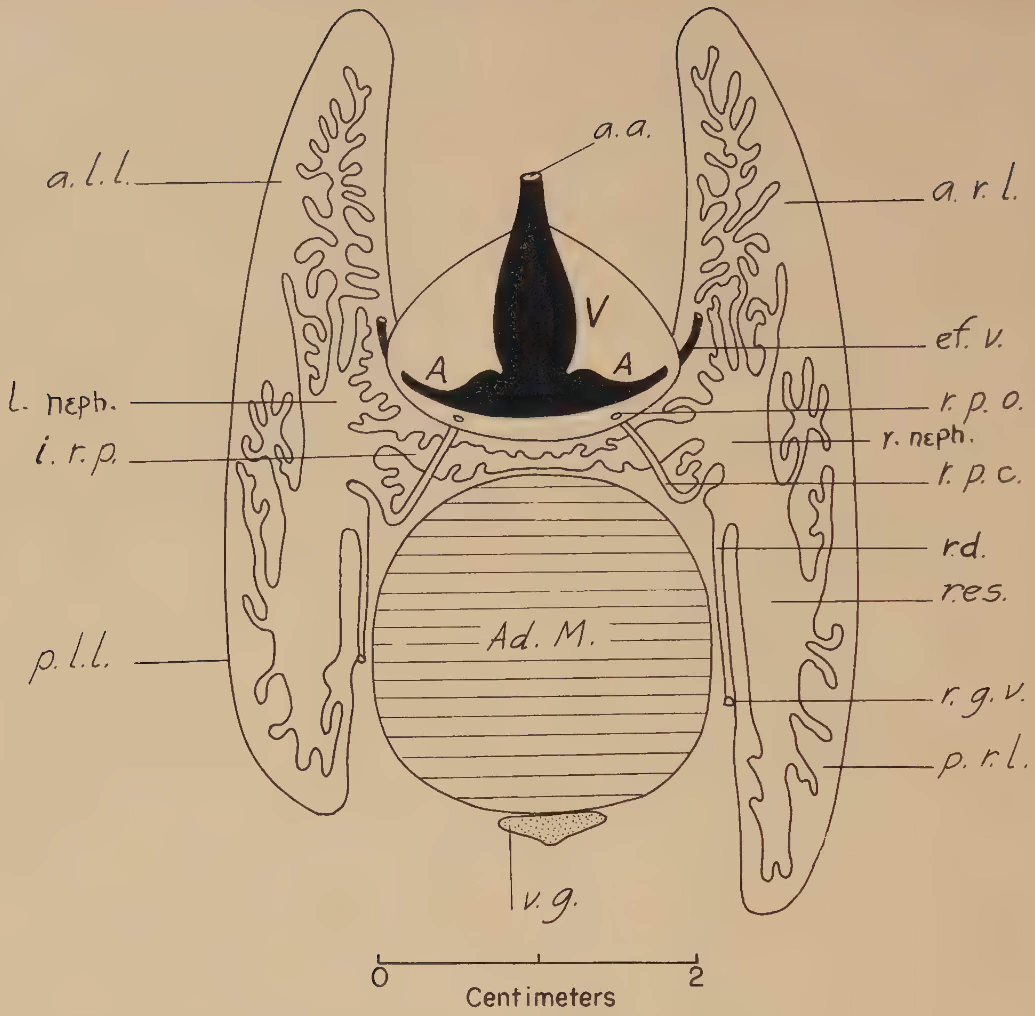

FIGURE 244.—Diagram of the excretory system of *C. virginica* based on examination of a series of transverse sections. Viewed from the anterior side. Gonads and their ducts, blood vessels, and blood sinuses are not shown. The pericardium wall is dissected and pulled down. A—auricle; a.a.—anterior aorta; Ad.M.—adductor muscle; a.l.l.—anterior left limb; a.r.l.—anterior right limb; ef.v.—efferent vein; i.r.p.—internephridial passage; l. neph.—left nephridium; p.l.l.—posterior left limb; p.r.l.—posterior right limb; r.d.—renal duct; r. neph.—right nephridium; res.—reservoir; r.g.v.—reno-gonadial vestibule; r.p.c.—reno-pericardial canal; r.p.o.—reno-pericardial opening; V—ventricle; v.g.—visceral ganglion.

by numerous branching and twisted tubules lined with excretory cells.

Much of the posterior limb of the kidney is occupied by a wide vesicle or reservoir (fig. 245, R.) for the storage of urine. A short renal duct (fig. 244, r.d., and fig. 247) leads from the reservoir to the outside and opens into the reno-gonadial vestibule, a shallow indentation on the surface of the pyloric caecum, behind the opening of the gonad (fig. 247). Numerous anastomosing tubules of the nephridia form a spongy tissue which extends into the visceral mass (fig. 248). Odhner (1912) pointed out that regardless of the apparent complexity of the nephridia, it is always possible to distinguish the two limbs or branches of the excretory system.

Topography and relationship of the excretory and reproductive systems vary greatly within the class of bivalves. Certain topographical features, however, remain constant. One of them is the position of the renal opening in relation to the cerebro-visceral connective. Lacaze-Duthiers (1855), one of the early students of molluscan anatomy, pointed out that the renal opening (also called "nephroprokt") is always located beyond the cerebro-visceral connective. Pelseneer (1891) at-

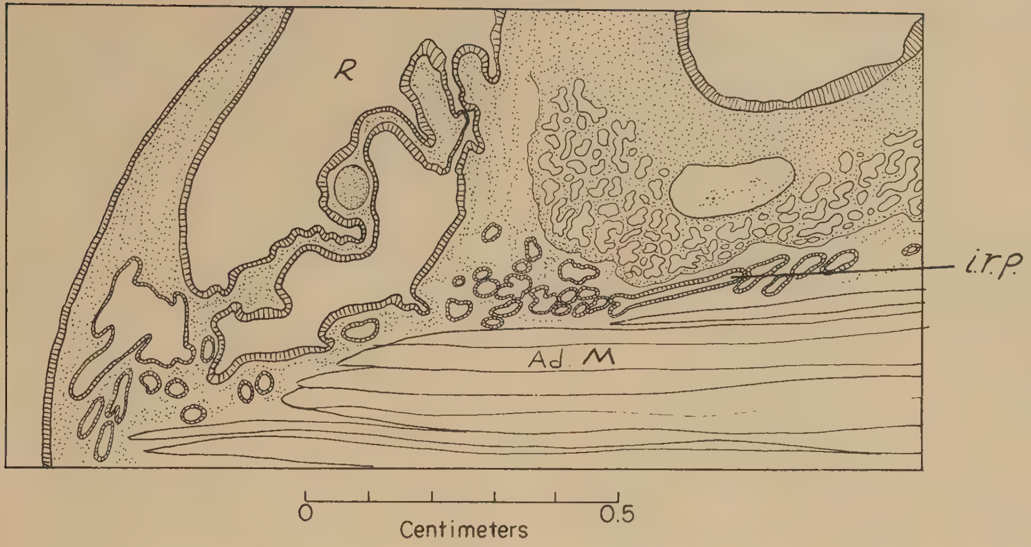

FIGURE 245.—Camera lucida drawing of a left portion of the central part of the excretory organ of *C. virginica* seen on cross section. The beginning of the inter-nephridial passage (i.r.p.) at the junction with the reservoir, R, is on the left side; Ad.M.—adductor muscle. Bouin, hematoxylin-eosin.

tached considerable importance to this morphological relationship for which he coined the term "ektaxial."

In the absence of a generally accepted anatomical terminology for the excretory system of bivalves, considerable confusion exists in the literature because of the great variety of names and descriptive terms applied to identical parts. The nephridium was discovered in 1817 by Bojanus (1819) who mistook it for the organ of respiration. Since then the excretory system of mollusks has been known in zoological literature as the organ of Bojanus. There seems to be no advantage in the continued use of this name, because the organ is frequently called kidney, nephridium, renopericardial or nephropericardial passage (Renopericardialgang, in German), and nephridial sac in cephalopods. The dorsal limb of the nephridium may be called the proximal part, inner limb, pericardial part, inner sac, excretional part, anteroposterior limb, and kidney sac. The ventral (or distal) limb is also known as outer sac, efferent part, and kidney passage. The reservoir is sometimes referred to as vesicle and bladder. The reno-pericardial opening is called nephrostome, nephridial funnel, and "Nierenspitze" or kidney syringe (Haas, 1935).

HISTOLOGY

The glandular nephridial tubes of the oyster are lined with a special epithelium; the cells are characterized by clear cytoplasm and absence of granules. Three kinds of epithelial cells are found in the different parts of the excretory system. In the tubules of the anterior limb the cells are of medium size, cylindrical, and vacuolated (fig. 249). The lining of the internephridial passage and of the tubules originating from its wall is made of short, almost cubical cells with inconspicuous vacuoles (fig. 250). In places the epithelium lining is two or three cells deep. Tall columnar cells with large terminal vacuoles are found primarily in the tubules of the posterior limb and in the reservoir (fig. 251 and fig. 252). The cells are about seven to nine times longer than their width. The vacuoles have no granules visible under the light microscope; they appear to contain liquid as if they are ready to burst. Some of the vacuoles seem to be empty, and many separated vacuoles are found in the lumen.

The lining of the reservoir consists of columnar cells of different heights; some of them are twice or three times taller than the others and protrude into the lumen (fig. 252). The epithelium rests on a basal membrane with a well-developed layer of circular muscles which extends to the renal duct. There is no organized sphincter for the control of the flow of urine. The contraction of the bladder in response to the fluid flowing into it from the pericardial cavity was observed by Fingerman and Fairbanks (1958).

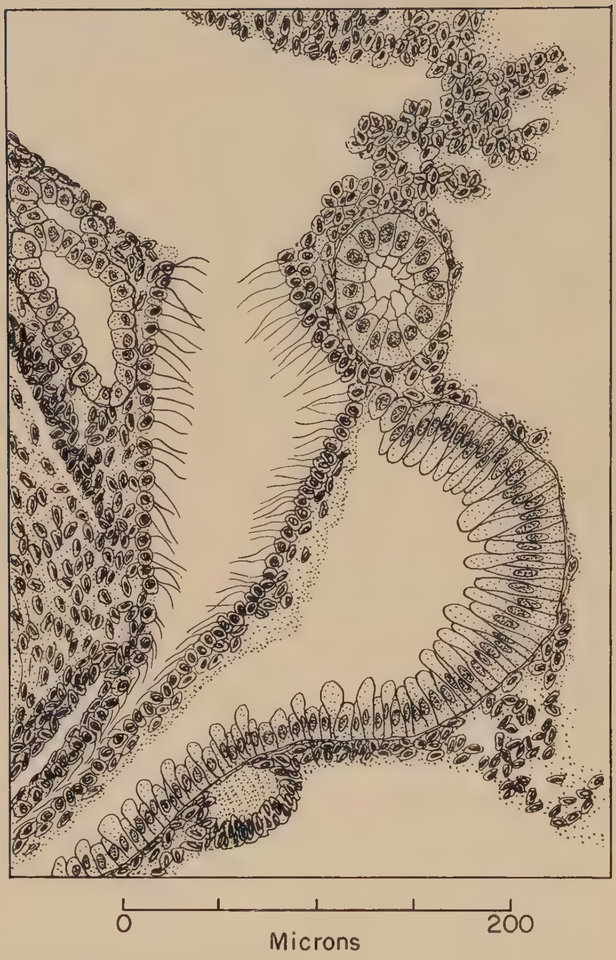

FIGURE 246.—Longitudinal section through the reno-pericardial opening and reno-pericardial canal, which connects the inner part of the kidney with the auricle in *C. virginica*. Part of the auricle at upper left and the adjacent portion of the kidney at lower right. Bouin, hematoxylin-eosin.

The epithelium of the reno-pericardial passage and of the renal duct is ciliated (fig. 246 and fig. 247).

PHYSIOLOGY

No comprehensive account of the biochemical and physiological processes of excretion in bivalves can be written at present because of the inadequate state of our knowledge, however, a review is possible.

During the past three-quarters of the century many studies have been made of the function of the organ of Bojanus and of the role of the pericardial glands. Most of these investigations were carried on for gastropods, cephalopods, and fresh-water mussels. The reader is referred to the review of this subject found in the papers of Marchal, 1889; Letellier, 1889; Cuénot, 1900; Fosse, 1913; Turchini, 1923; Delaunay, 1924, 1931, 1934; Spitzer, 1937; and Franc, 1960. Very little work has been done, however, with marine bivalves and particularly with oysters for which physiological studies of the excretory system present considerable technical difficulties. The nephridial organs of oysters do not form a sharply outlined unit. They are surrounded by delicate and loose connective tissue which contains numerous blood vessels with very thin walls and spacious sinuses. Consequently, it is very difficult to obtain a sample of the liquid from the various parts of the kidney system without contaminating it with blood or with the sea water which surrounds the organ. Such difficulties are not encountered in the studies of land snails and cephalopods.

An experimental approach to the study of the function of nephridia was made by Grobben (1888), Kowalevsky (1890), and Emeljanenko (1910) who injected various dyes in the foot, mantle, or visceral mass of the mollusk. It was shown by this method that the excretory organs of bivalves excrete indigo-carmine and that ammonia carmine injected in physiological solution is concentrated in the pericardial glands. In *Ostrea* the carminates are concentrated in the walls of the auricles, while in *Pecten maximus* they are spread throughout the connective tissue (Cuénot, 1900).

Attempts to determine the rate of filtration of fluid into the pericardium of fresh-water Unionidae were made by Turchini (1923) and Picken (1937). Picken studied the hydrostatic and colloid osmotic pressure of the body fluid. He supposed, probably correctly, that the pericardial fluid is a filtrate of the blood through the wall of the heart. His observation that the pericardial fluid of *Anodonta* is isotonic with blood seems to support this view.

On the basis of the experiments by these authors and by Robertson (1949, 1953), the following course of urine formation may be visualized: The blood is filtered through the wall of the heart into the pericardium, and this fluid, together with the secretion of the pericardial glands, passes through the reno-pericardial opening into the nephridial tubes. The secretion from the tubules is added to the liquid. Its final composition will depend on how far and how rapidly the secretion and possibly the reabsorption of certain constituents proceed in the secretory epithelium.

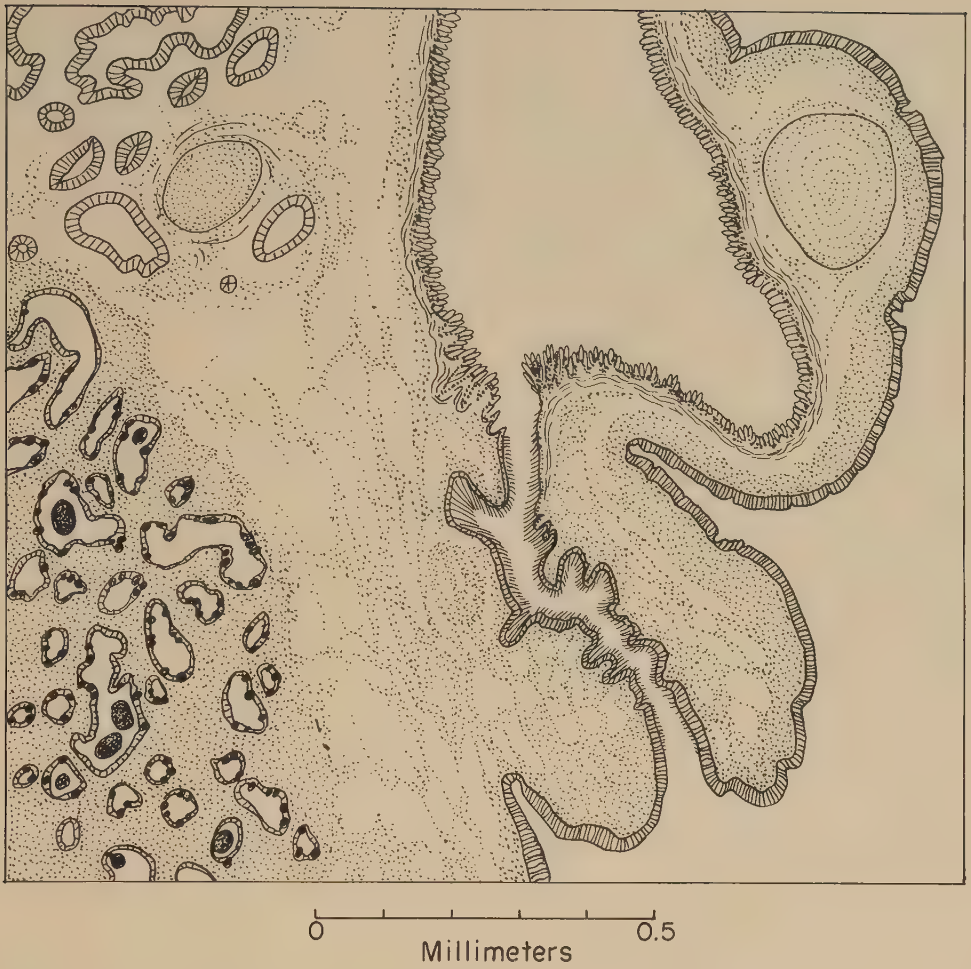

FIGURE 247.—Section through the reservoir and renal duct of the right nephridium *C. virginica*. Gonad at the lower left corner and cerebro-visceral connective at the upper right corner. Bouin, hematoxylin–eosin.

The experimental procedures for separate collection of blood, pericardial fluid, and urine from the reservoir are uncertain, both because the tissues are fragile and because a rich supply of blood is received by the nephridia directly from the visceral sinuses. Prosser (1950, p. 36) states correctly that urine volume cannot, therefore, be calculated from the rate of accumulation of the pericardial fluid; neither can the composition of urine be inferred from an analysis of the fluid collected from the interior of the kidney.

The rate of filtration of blood from the ventricular wall into the pericardium was studied in *Anodonta* by Picken (1937) using the following method: A hypodermic needle of 1.5 mm. bore was inserted in the pericardial wall previously exposed by cutting off the adjacent portion of the shell. A length of about 2 cm. of glass tubing was sealed to the hypodermic needle, which was tilted so that there was a difference of about 0.5 cm. in the levels at each end of the needle. The fluid from the pericardium dropping through the needle was collected for periods of 5 minutes at 10-minute intervals. The average rate of filtration determined in this manner was about 25 ml. in 4 hours. The method probably gives a higher rate of blood filtration than occurs under normal conditions because of a loss of pressure inside the pericardium resulting from the puncture of the wall. Furthermore, it does not necessarily follow that the rate of the discharge of fluid by the kidney is controlled by the rate at which the pericardium fills up with blood. The method does not seem applicable to the oyster because of the position of the excretory organs on the oyster body and the inherent technical difficulties.

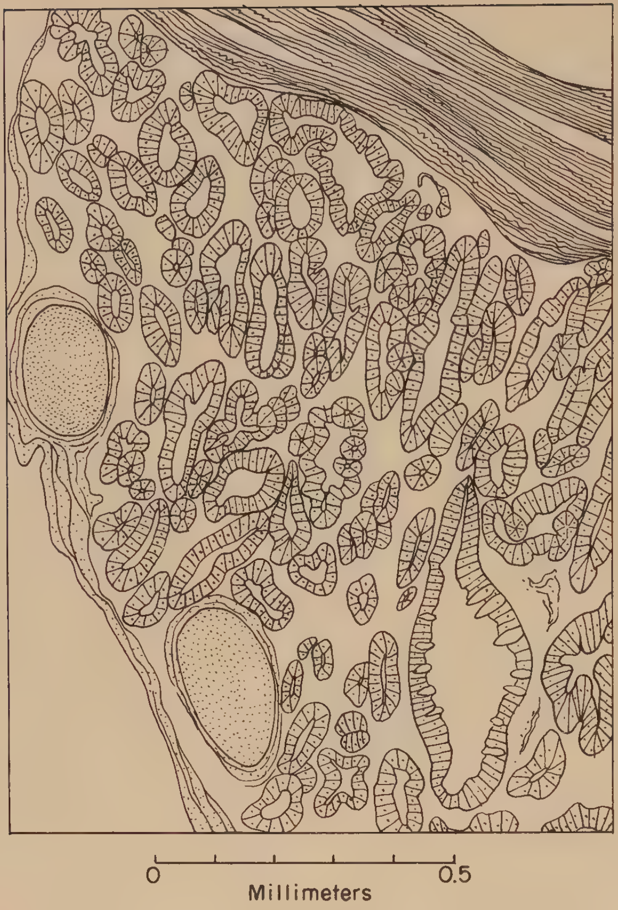

FIGURE 248.—Tightly packed and twisted blind tubules of the excretory organ of *C. virginica* form "spongy" tissue which extends on both sides into the visceral mass. Adductor muscle at upper right. Bouin, hematoxylin–eosin.

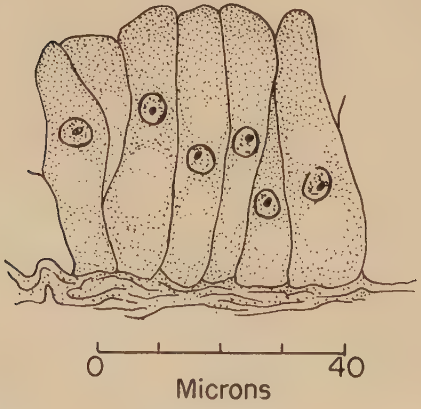

FIGURE 249.—Medium-sized cylindrical epithelial cells lining the lumen of the nephridial tubules of the anterior limb of *C. virginica*. Oil immersion. Kahle, hematoxylin–eosin.

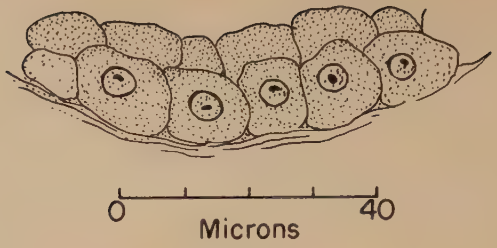

FIGURE 250.—Cubical cells of the epithelium lining of the inner wall of the internephridial canal of *C. virginica*. Oil immersion. Kahle, hematoxylin–eosin.

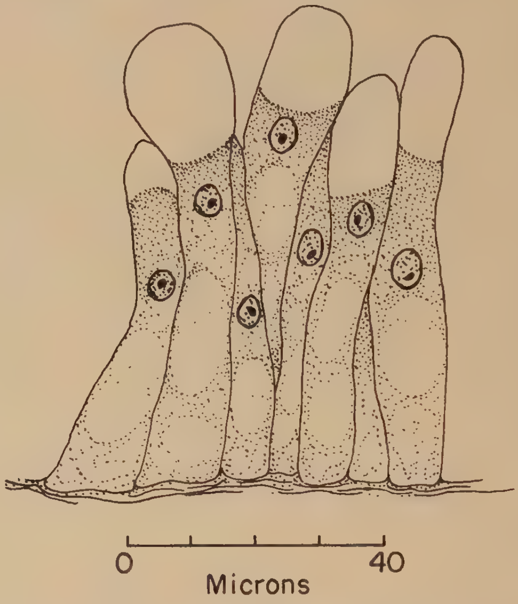

FIGURE 251.—Tall columnar cells of the lining of the tubules of the posterior limb of *C. virginica*. Oil immersion. Kahle, hematoxylin–eosin.

THE WASTE PRODUCTS

Relatively little is known about nitrogen excretion in the oyster or in other marine bivalves. As in other organisms the waste products are various nitrogenous compounds. The presence of a low concentration of ammonia, the principal nitrogenous product of amino acid breakdown in mollusks (0.051 mg. per 100 ml.) was demonstrated by Florkin and Houet (1938). In the excreta of the marine clam (*Mya arenaria*) ammonia comprises 21.5 percent of the total nonprotein nitrogen (Delaunay, 1924), and in *Mytilus* the figure varies from traces to 10.8 percent (Spitzer, 1937). Small quantities of urea amounting to 4.5 percent of the nonprotein nitrogen excreted in different forms were found in *Mya* and only traces of it in *Mytilus*. No uric acid was found in the latter species

(Spitzer, 1937) and only traces were reported in *Mya*. In both organisms amino acids in the excreted material amounted to 18 percent in *Mya* and from 17 to 35 percent in *Mytilus*.

Two different methods were used in the studies of nitrogen secretion. The nephridia with adjacent tissues were ground with sand and extracted with distilled water, and the extracts analyzed; alternately, the entire animals were placed in small quantities of distilled water, kept at 16° to 18° C. for a period of time varying from 24 to 72 hours, and the metabolites accumulated in the water were analyzed. It appears from the experiments in which both fresh-water and marine bivalves were used that uric acid is not present in the nephridia of *Anodonta*, *Unio*, *Mytilus*, and *Mya* (Spitzer, 1937; Przylecki, 1922a). Przylecki found that in *Anodonta* up to 60 percent of the total excreted nitrogen is represented by ammonia, and that the amount of ammonia excretion may be greatly increased by placing the animals in acidified water for a short time. He also found that urease, the enzyme which converts urea into ammonia, is present in abundance in *Mytilus edulis* and *Helix* (Przylecki, 1922b). In a study of the urinary functions in bivalves Letellier (1887, 1889) arrived at the conclusion that in the nephridia of *Mytilus*, *Anodonta*, and *Cardium* urea takes the place of the uric acid; and Marchal (1889) was not able to find uric acid in the excretion of 50 mussels he tested. Spitzer (1937) demonstrated, however, the presence of uric acid in the middle intestine of *Unio* and *Mytilus*. Delaunay (1931) found traces of uric acid in *Mya arenaria* and small quantities of it in the products of nitrogen excretion in the Portuguese oyster *Crassostrea* (*Gryphaea*) *angulata*.

Among the identified nitrogenous metabolites excreted by *Mya arenaria* and *Mytilus edulis* the largest proportion belongs to the amino acids. Next in importance is ammonia, followed by purine, urea, and uric acid. The numerical values expressed in grams of nitrogen of a given compound per total nitrogen in excreta are given in table 32.

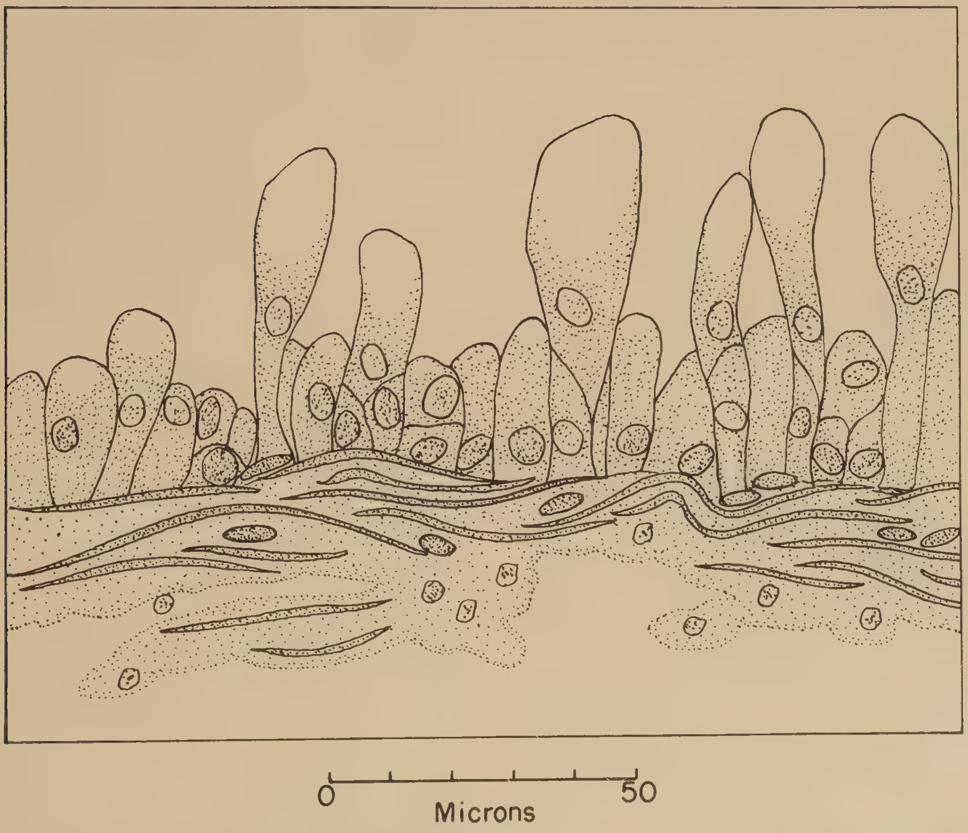

FIGURE 252.—Columnar cells of the lining of the kidney reservoir of *C. virginica*. Basal membrane with a well-developed layer of circular muscles. Oil immersion. Kahle, hematoxylin–eosin.

Baldwin (1935) suggested that the enzyme arginase may be involved in the elaboration of both urea and uric acid in gastropods. He found no evidence of its presence in *Mytilus edulis* or *Pecten opercularis* although the enzyme was found in fresh-water *Anodonta*. Brunel (1938) points out that many investigations have shown that uric acid and allantoine (the product of oxidation of uric acid) are not found in bivalves, and Needham (1935) considers that its absence in that class and its presence in land snails are adaptations to terrestrial life by uricotelic organisms, which often can not find sufficient water for their needs and avoid toxemia by converting poisonous ammonia into the insoluble and relatively innocuous uric acid. The body of a marine bivalve is, as a rule, permeable to water and to small molecules. Consequently, the ammonia formed during catabolism easily escapes by diffusion into the external environment.

TABLE 32.—*Excretion of nitrogen in three species of bivalves in grams of nitrogen per total nitrogen excreted*

[From Handbook of biological data, 1956, Spector, ed., p. 43]

Species	Amino acid N	Ammonia N	Purine N	Urea N	Uric acid N	Unidentified N
Crassostrea angulata	13.2	7.2		3.2	1.6	75
Mya arenaria	18	21.5	5	4.3	Trace	51
Mytilus edulis	17–36	Trace-11.4	Trace-16	Trace		39–79

OSMOREGULATION

It is generally considered that marine bivalves have little power of osmoregulation when placed in diluted sea water (Morton, 1958), and can prevent loss of salts only by closing their valves. There is, however, an indication that the excretory system may serve as an osmoregulating organ. Kumano (1929) reported almost complete agreement between the ionic concentrations of the blood and pericardial fluid of *O. circumpicta* and the sea water in which the oyster was kept. The exception was reported only for magnesium and sulfate ions, which were higher in the blood by 11 and 7 percent respectively than in the sea water. The corresponding values for the pericardial fluid were 5 percent higher for magnesium and 2 percent lower for sulfate.

Robertson (1949, 1953) found that marine bivalves and gastropods (*Pecten*, *Mya*, *Ensis*, *Pleurobranchus*, *Neptunea*) accumulate potassium and calcium and eliminate sulphate to a small degree. The range of values expressed in percentage of ionic concentration in the surrounding sea water was as follows: sodium, 97 to 101; calcium, 103 to 112; magnesium, 97 to 103; chlorine, 99 to 101; sulphate, 87 to 102.

Robertson defined ionic regulation as the maintenance of ionic concentrations in the body fluid differing from those found in a passive equilibrium with the external medium. Accordingly, he made an analysis of a body fluid obtained from a mollusk and determined its ionic concentration. A second determination was made using the same fluid after it had been dialyzed in a collodion sac against the original sea water. The results reproduced in table 33 show that *Mytilus* and *Ostrea* exert little ionic regulation in their coelomic fluid or plasma, apart from the accumulation of potassium. Magnesium remains within 3 to 4 percent of the equilibrium while calcium sometimes exceeds this by a few percent. The accumulation of sulphates by *Mytilus galloprovincialis* was regarded by Robertson as a rare feature in marine invertebrates. The protein content of blood plasma was found to be low (0.2 to 0.3 percent) in *O. edulis* and significantly higher in *M. galloprovincialis*.

TABLE 33.—*Ionic concentrations in plasma as percentage of concentrations in dialyzed plasma (according to Robertson, 1953)*

[Data based on pooled samples]

	Na	K	Ca	Mg	Cl	SO_4	Protein mg./ml.
Ostrea edulis	99.5	129.1	100.6	102.3	99.8	99.7	0.2
Mytilus edulis	100.0	134.7	99.5	99.5	100.5	98.2	0.3
M. galloprovincialis	100.9	120.8	106.6	96.5	98.6	120.0	0.8

The suggestion that some sort of osmoregulation is present in *C. virginica* was made by Fingerman and Fairbanks (1957, 1958), who placed Louisiana oysters in sea water of three different salinities. After periods up to 14 days, samples were taken from the ventricles, the pericardial cavity, the reservoir of the excretory system, the secretory portion of the nephridia, and the mantle. The authors claim that at the highest concentration of 515.6 milliequivalents of chloride per l. all fluids were hypotonic and at low salinities (161.7 milliequivalents of chloride per l.) all body fluids were hypertonic to the environment. The original data are not given, and the results are presented as average differences. The "t" test of significance ranging in the values of P from 0.20 to 0.50 seems to indicate that the differences are not significant. The authors' conclusion can not, therefore, be accepted without further confirmation.

The relative significance of the nephridia, pericardial glands, and phagocytes in excretion can not be evaluated at present. The three different systems probably supplement one another by eliminating different waste products. Histochemical studies show, for instance, that phagocytes play the major role in the accumulation, storage, and discharge of iron and copper. Undoubtedly the phagocytes heavily loaded with dark granules contain other substances besides heavy metals which are discarded by the organism. On the other hand, other tissues also are involved in the process of excretion. For instance, the epithelia of the gills, of the edge of the mantle, and of the labial palps under certain conditions store iron. My observations on *C. virginica* indicate that iron is excreted by the epithelium of the mantle, and Stauber (1950) found that India ink injected into the ventricle is distributed by the phagocytes to all parts of the organism and is eliminated through the epithelium of the alimentary tract, digestive diverticula, palps, mantle, and pericardium. Nephridia and shell-forming parts of the mantle are not, however, the routes of migration of these cells. Observations made by Kowalevsky (1890) and Cuénot (1900) showed that if a mollusk with pericardial glands is injected with a mixture of indigo carmine and ammonia carmine, the latter is concentrated in the cells of the pericardial glands, while the indigo goes into the nephridia. These separations lead some investigators to designate the different cells as "carmineathrocytes" and "indigoathrocytes" (Strohl, 1924), terms which have not been accepted by biologists. The affinity of these cells to special dyes does not necessarily indicate that they participate in the normal process of excretion.

Pigmentation may be considered as a certain phase of excretion. Green oysters of Long Island Sound develop dark green pigment as the result of absorption and storage of copper by the phagocytes (Galtsoff and Whipple, 1931). Schiedt (1904) considered that the development of black pigment in the mantle of oysters exposed to strong illumination is also a product of excretion. His finding was not in agreement with the observations of Faussek (1899) on pigmentation on the mantle of *Mytilus* with partially removed shells.

Excretion through diapedesis is undoubtedly of great importance to the mollusk, but its exact role is not fully understood.

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CHAPTER XIII

THE NERVOUS SYSTEM

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The nervous system of the oyster is relatively simple. The visceral and cerebral ganglia are joined by the cerebro-visceral connectives; the U-shaped cerebral commissure goes around the esophagus; the circumpallial nerve travels along the mantle's edge; and a number of nerves originate from the ganglia and extend to different parts of the body. The pedal ganglia, well developed in many other bivalves, and the cerebropedal connective are absent. These retrogressive features are associated with the sedentary mode of life of the oyster and the loss of the organ of locomotion (foot). In the evolution of bivalves this simplification of the anatomy represents an adaptive change and cannot be regarded as a primitive trait (Jhering, 1877).

The only organs of sense in the oyster are the tentacles, along the edge of the mantle, and the pallial organ inside the cloaca. The tentacles are highly sensitive to changes in illumination; they contract if a shadow passes in front of a feeding oyster, or a beam of light is focused on them. They also detect the presence of minute quantities of various drugs, chemicals, excessive amounts of suspended particles, and changes in temperature and composition of sea water. The function of the pallial organ is not well understood; the organ is probably concerned with the detection of mechanical disturbances in the surrounding water.

The eyes found in freely moving bivalves, such as scallops, are absent in adult oysters but are present in fully grown larvae.

There is no major control center (brain), and in this sense the nervous system is decentralized. The integration of its various parts is accomplished by the interconnections of the ganglia through the cerebro-visceral connectives, cerebral commissure, and the larger nerve trunks. All these parts contain groups or nuclei of nerve cells and have a structure similar to that of the ganglia.

In oysters the two visceral ganglia are fused into a single organ. Its double origin is clearly visible on tangential sections.

The distribution of nerves in bivalves was studied by many zoologists of the 19th century (Garner, 1837; Duvernoy, 1854; Jhering, 1877; Rawitz, 1887; Babor, 1896; Pelseneer, 1891; Freidenfelt, 1896; Gilchrist, 1898) who described the principal topographic features of the nervous system of various species. The nerves of the European oysters were quite accurately depicted in the old paper of Duvernoy; a general description of the nerve system of *O. chilensis* was given by Dahmen (1923); of *C. angulata* by Leenhardt (1926), and of *O. cucullata* by Awati and Rai (1931).

METHODS

Anatomical dissection of the nervous system of the oyster is rather difficult. The nerves are small, unpigmented, and are embedded in connective tissue. In fully ripe or in so-called "fat" oysters even the principal nerves of the visceral mass are hidden under a thick layer of gonad or are covered by large quantities of glycogen. The lean and watery specimens usually found shortly after spawning are most suitable for dissection. Immersion in 10 percent nitric acid, followed by washing and clarification in glycerol, may be useful because the nerve tissue is stained by the acid a light brown color. The entire nervous system may be stained in toto by using the following procedure: oyster tissue preserved in 95 percent ethyl alcohol or in 5 percent formalin is transferred into 1 percent aqueous solution of potassium hydroxide (solution No. 1) for 1 to 3 days. The specimen is then placed in solution No. 2 made of 1 part glacial acetic acid, 1 part glycerol and 6 parts of 1 percent chloral hydrate solution, and is left in it for about 24 hours. The tissue is then transferred into solution No. 3 consisting of 1 part of Ehrlich acid hematoxylin, 1 part of glycerol and 6 parts of 1 percent chloral hydrate solution. After several days in this mixture the preparation is destained in the solution No. 2 for about 12 to 24 hours and cleared in glycerol. Time of staining and destaining may be modified depending on thickness and condition of tissues. In successful preparations the dark purple nervous tissue is visible against the semitransparent visceral mass. In my experience the method proved to be capricious and not entirely reliable.

In a live oyster the nerves appear as thin, white threads which can be traced for some distance in very thin oysters containing no glycogen. Oysters starved for 4 to 6 weeks in filtered sea water were found to be very suitable for nerve study. Dissection of the nervous system must be supplemented by reconstruction of ganglia and nerves through examination of serially sectioned material.

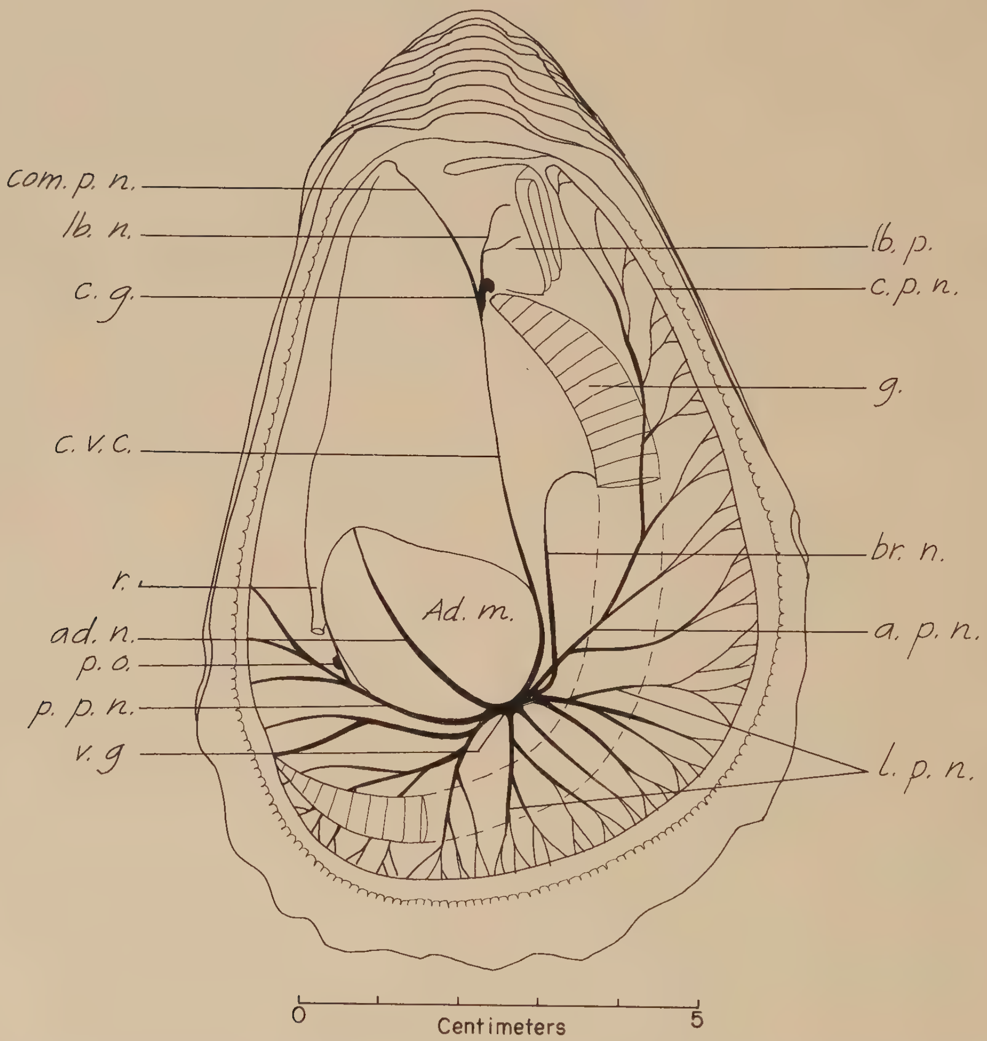

FIGURE 253.—Diagram of the nervous system of *C. virginica* seen from the right side. Dorsal and ventral ends of the gills are shown under the mantle; the middle portion of the gill has been cut off; the middle portion of the branchial nerve is indicated by the dotted line. Ad.m.—adductor muscle; ad.n.—adductor muscle nerve; a.p.n.—anterior pallial nerve; br.n.—branchial nerve; c.g.—cerebral ganglion; c.p.n.—circumpallial nerve; com.p.n.—common pallial nerve; c.v.c.—cerebro-visceral connective; g.—gills; lb.n.—labial nerve; lb.p.—labial palps; l.p.n.—lateral pallial nerves; p.o.—pallial organ; p.p.n.—posterior pallial nerve; r.—rectum; v.g.—visceral ganglion. The cerebral commissure is not visible.

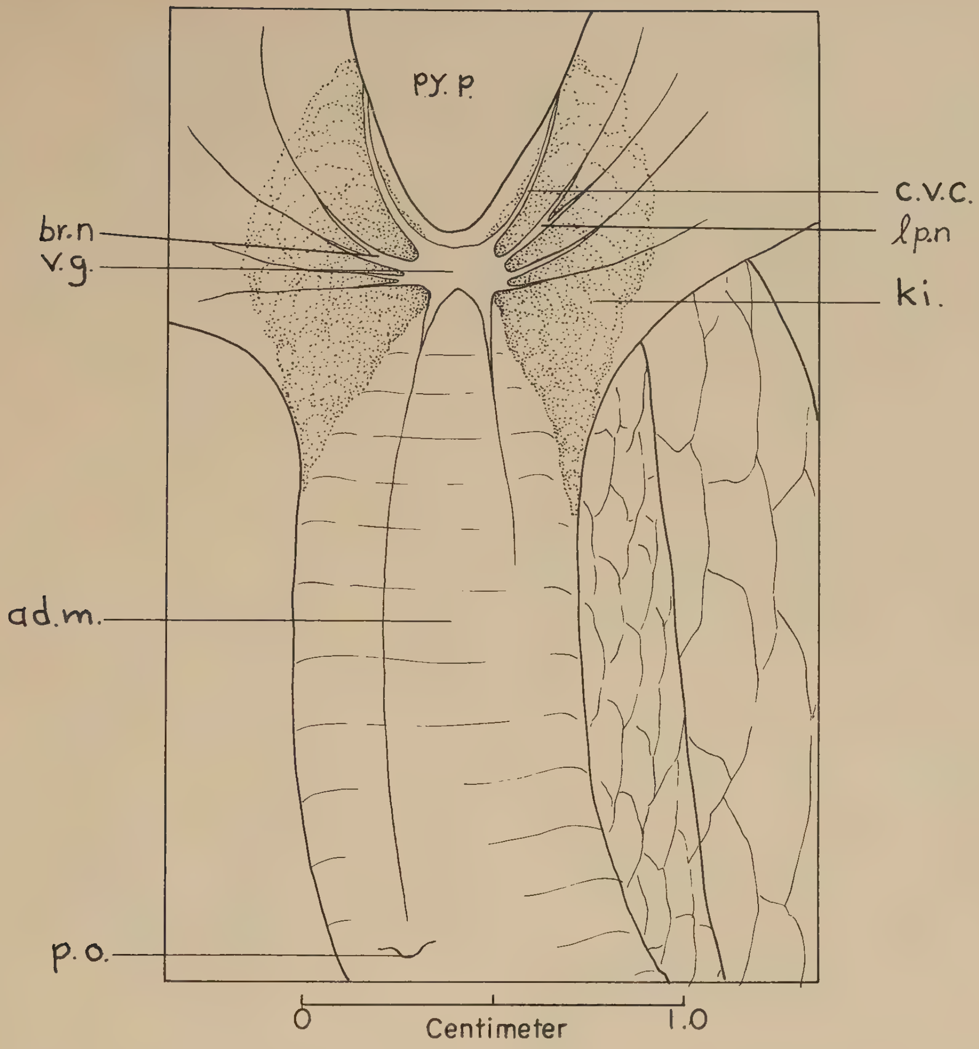

FIGURE 254.—Visceral ganglion of a large *C. virginica* seen through the epibranchial chamber. ad.m.—ventral side of the adductor muscle; br.n.—branchial nerve; c.v.c.—cerebro-visceral connective; ki.—kidney; l.p.n.—labial palp nerve; p.o.—pallial organ; py.p.—pyloric process; v.g.—visceral ganglion. The oyster was fully narcotized and the adductor muscle completely relaxed. Drawn from life.

ANATOMY

The visceral ganglion, the largest unit of the nervous system, is a wedge-shaped structure embedded on the anteroventral side of the adductor muscle in a depression formed by the junction of the translucent and opaque parts of the muscle. To see the ganglion in its natural position one must cut off the wall of the epibranchial chamber and lift the tip of the pyloric process. The location of the ganglion in such a preparation, examined from the right side of the oyster, is shown in figure 253. The entire ganglion can be examined in situ in an intact oyster. For this purpose the oyster is narcotized until the valves gape, and a beam of light is directed into its cloaca with the oyster's posteroventral end held toward the observer; when the pyloric process is raised slightly with a probe, the ganglion becomes visible as a white or sometimes slightly yellowish flat organ (fig. 254). In the preparation from which the drawing was made the muscle was completely relaxed and the gaping distance between the valves measured about one-half inch.

The visceral ganglion is highly developed in all Ostreaceae (Rawitz, 1887). The right and left components, fused into a single organ, are distinguishable, and the ganglion appears to consist of three parts, one central section and the two lateral sections from which emerge six pairs of nerves. In some bivalves the ganglion and the nerves are pigmented, but not in the oyster.

The pair of cerebral ganglia of the oyster (fig. 253, c.g.) is located at the bases of the labial palps. The position of these small organs is slightly asymmetrical in relation to the palps, with the left ganglion slightly lower than the right one. Because of such asymmetry, only one ganglion is usually seen in the transverse sections of the visceral mass made at the level of the bases of the palps. In large and thin ("poor") oysters the cerebral ganglia may easily be located, but in "fat" specimens they are not usually clearly visible.

The narrow cerebral ganglion of each side is bent at a sharp angle: this gives it the appearance of a saddle sitting over the basal membrane of the epithelium of the palps (fig. 255). The cerebral commissure emerges from the end of the ganglion (c.com.) and makes an inverted U-shaped loop which passes dorsally over the esophagus and connects with the ganglion of the opposite side.

The following nerves emerge from the anterior side of the visceral ganglion: The cerebro-visceral connectives rise from the anterior side of the ganglion and soon are buried in the connective tissue of the visceral mass (figs. 253, 254, 255, c.v.c.); next to the connectives and slightly dorsal to their roots are the anterior pallial nerves (a.p.n.), which run forward through the kidney, pass across the pericardium wall toward the dorsal part of the body and along their course give off branches extending to the central part of the mantle. Two branchial nerves (figs. 253, 254, br.n.), arise near the roots of the cerebro-visceral connective and run parallel to it for a short distance. These nerves are more easily recognizable than the others because they form a convex curve and enter the gill axis on each side of the body accompanied by the efferent blood vessels. The two distinct branches of the lateral pallial nerves originate from the extreme points on the sides of the visceral ganglion. The outer branch (upper in fig. 254) almost immediately enters the mantle, while the inner branch (lower in fig. 254) divides into numerous smaller nerves which establish contact with the circumpallial nerve (fig. 253, c.p.n.). The posterior pallial nerve (fig. 253, 255, p.p.n.) runs from the ventral end of the ganglion along the ventral side of the adductor muscle as far as the rectum and divides into smaller branches which penetrate the mantle. The pallial organ (fig. 255, p.o.) is located along the course of this nerve. A pair of adductor nerves arises from the dorsal side of the ganglion and immediately enters the muscle tissue (fig. 255, ad.n.). They are not visible from the ventral side of the muscle.

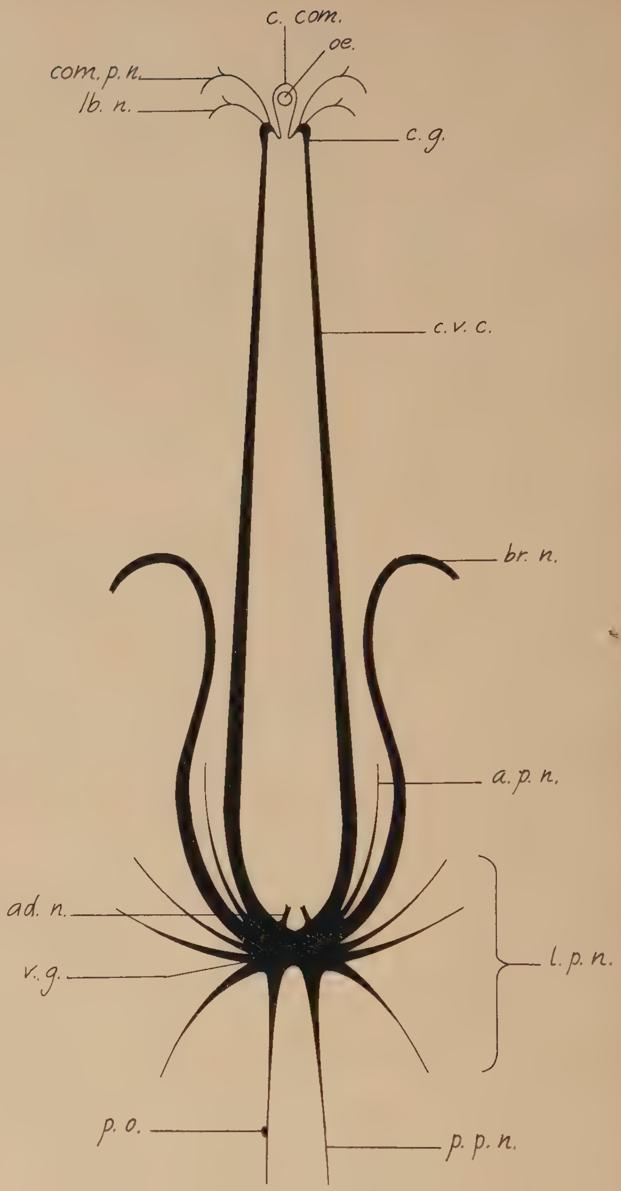

Figure 255.—Diagram of the nervous system of *C. virginica* seen from the anterior side. ad.n.—adductor muscle nerve; a.p.n.—anterior pallial nerve; br. n.—branchial nerve; c.g.—cerebral ganglion; c. com.—cerebral commissure; com.p.n.—common pallial nerve; c.v.c.—cerebro-visceral connective; lb.n.—labial nerve; l.p.n.—lateral pallial nerves; oe.—esophagus; p.o.—pallial organ; p.p.n.—posterior pallial nerve; v.g.—visceral ganglion.

Except for the pair of cerebro-visceral connectives, only a few nerve trunks originate from the cerebral ganglia. The common pallial nerve (figs. 253 and 255, com.p.n.) enters the mantle of the corresponding side and establishes connection with the dorsal portion of the circumpallial nerve. The labial nerves (lb.n.) branch to the four labial palps.

The circumpallial nerve (fig. 253, c.p.n.) is a fairly large nerve trunk which runs parallel to the edge of the mantle. Throughout its course it is accompanied by the circumpallial artery and supplies numerous branches to the tentacles and makes connections to the radial nerves which extend from the base of the mantle to its edge (see: fig. 86 in chapter V). The circumpallial nerve is connected anteriorly with the cerebral ganglia and posteriorly ends in the visceral ganglion. Stimuli received anywhere on the mantle may be transmitted to the entire nervous system of the oyster by this circular nerve. Because of this arrangement the oyster may respond to stimuli as a whole in spite of separation of its nerve centers.

The cerebral and visceral ganglia also are joined by a pair of relatively broad and long cerebro-visceral connectives (fig. 253, 255, c.v.c.), which constitute the main nerve trunks through which communication is maintained with all the parts of the body.

MICROSCOPIC STRUCTURE

The ganglia are formed of a central core or the neuropile, tightly packed bundles of nerve fibers, and the cortex made of several layers of nerve cells. This arrangement gives the ganglia a resemblance to the white and gray matter of the central nervous system of vertebrates. A layer of loose connective tissue forms the outer sheath of the molluscan ganglia.

The visceral ganglion is a relatively large, wedge-shaped structure embedded on the ventral side of the adductor muscle in the depression between its two parts (fig. 256). In the oyster the ganglion is completely fused but its paired origin is clearly seen on a tangential section (fig. 257).

The cortex is made of a continuous layer of nerve cells. Scattered nerve cells also occur in the neuropile. The ventral side of the ganglion facing the epibranchial chamber is covered with a unicellular layer of epithelium.

The nerve cells which in the oyster and other bivalves form the cortex were described by Rawitz (1887), whose paper remains the major contribution to the histology of the molluscan nervous system. To obtain the entire nerve cells with their axons Rawitz macerated small pieces of ganglia in 25 percent ethyl alcohol for 4 to 5 hours or in an aqueous solution of potassium bichromate (from 0.025 to 0.1 percent) for 8 to 24 hours.

Individual nerve cells of the oyster are either pear- or club-shaped with one, two, or several processes extending from their bodies. These processes give rise to fine fibrillae which enter the neuropile. Depending on the number of the processes, the cells are called unipolar (fig. 258, a, b), bipolar (e), and multipolar (c, d). The unipolar cells are more abundant than the other two types. The apolar cells, i.e., those without the processes, have not been found in bivalves, according to Rawitz.

The size of nerve cells varies. The multipolar cells are usually the largest; their dimensions, without the processes, range from 14.5 μ by 5 μ to 20 μ by 8 μ. The unipolar and bipolar cells are smaller, varying in size from 12.5 μ by 4 μ to 14 μ by 6 μ. The tapered ends of the cells give rise to the nerve fibers, which enter the neuropile where they combine with other nerve fibers to form several compact bundles. Single bipolar and unipolar cells are scattered throughout the neuropile, but do not aggregate into distinct nuclei or groups. The protoplasm of the nerve cell is dense and is deeply stained with Ehrlich's and Delafield hematoxylin.

The nerve cells and their axons are supported by a framework of connective tissue cells which descend from an outer sheath of the ganglion. These cells were observed first by Freidenfelt (1897). Bochenek (1906) and Jakubski (1912, 1913) described the supporting elements in the ganglia of *Anodonta*, *Pinna*, and several gastropods, tunicates, and echinoderms, and regarded them as typical glia cells. Jakubski distinguished three groups of glia cells: (1) Star-shaped flat cells with oval nuclei and thin processes which continue as neuroglia fibers were found in the outer sheath of the ganglion, (2) spindle-shaped cells with a pointed nucleus usually have two outgrowths and are most common in the inner portion of the ganglion for which they form a supporting framework; and (3) "neuropile glia cells", which occur singly, scattered through the neuropile. Jakubski

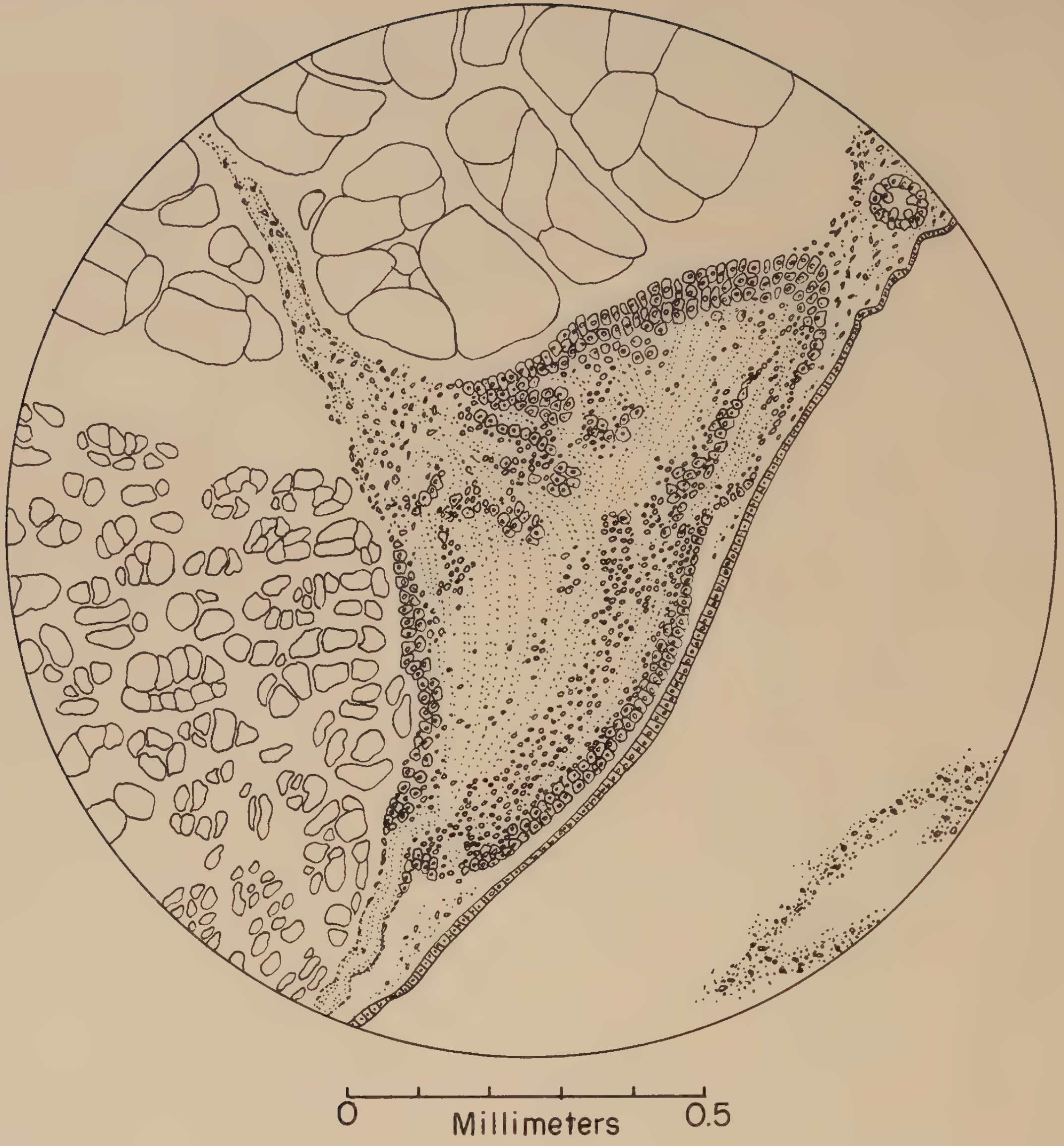

FIGURE 256.—Visceral ganglion of *C. virginica*. Transverse section at the right angle to the fibers of the adductor muscle. Bouin, hematoxylin-eosin.

(1912) pointed out that the interstitial elements of the neuropile do not isolate the nervous elements, while in the nerves and in the commissures the glia cells surround a number of nerve fibrillae which form distinct tracts. Of the three different types of glia cells described by Jakubski, the narrow, spindle-shaped cells could be seen in the preparation of the ganglia of *C. virginica* stained with Delafield and iron hematoxylin. Nissl granules were described in the multi-polar nerve cells at the ends of siphons in *Mya* (Piéron, 1941).

The nerve fibers which form the neuropile are clearly noticeable in sections of the visceral ganglion (fig. 256); some of them cross the ganglion, while others enter the viscero-cerebral connective on the same side where they emerged.

Rawitz (1887) described in detail the pathways of these fibers in the visceral ganglion of *Mytilus*. He found that in each half of the ganglion approximately one-quarter of the fibers originates from the nerve cells of the same section; one-quarter arises from the opposite half; one-quarter is derived from the cerebro-visceral connective of the corresponding side; and one-quarter originates in the cerebro-visceral connective of the opposite side. It is difficult to determine these pathways in the

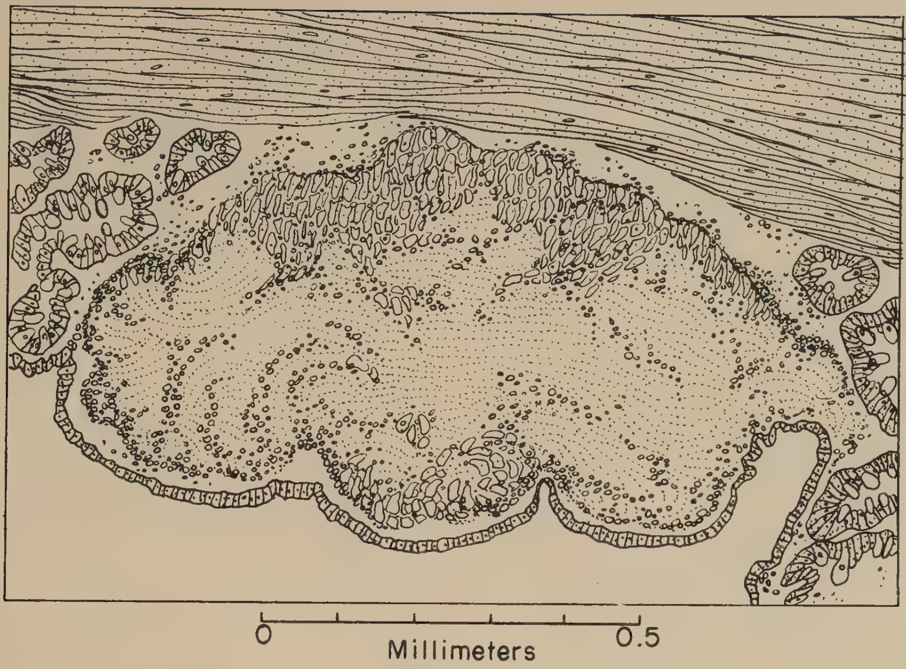
0
Millimeters
0.5

FIGURE 257.—Visceral ganglion of *C. virginica*. Tangential section. Bouin, hematoxylin-eosin.

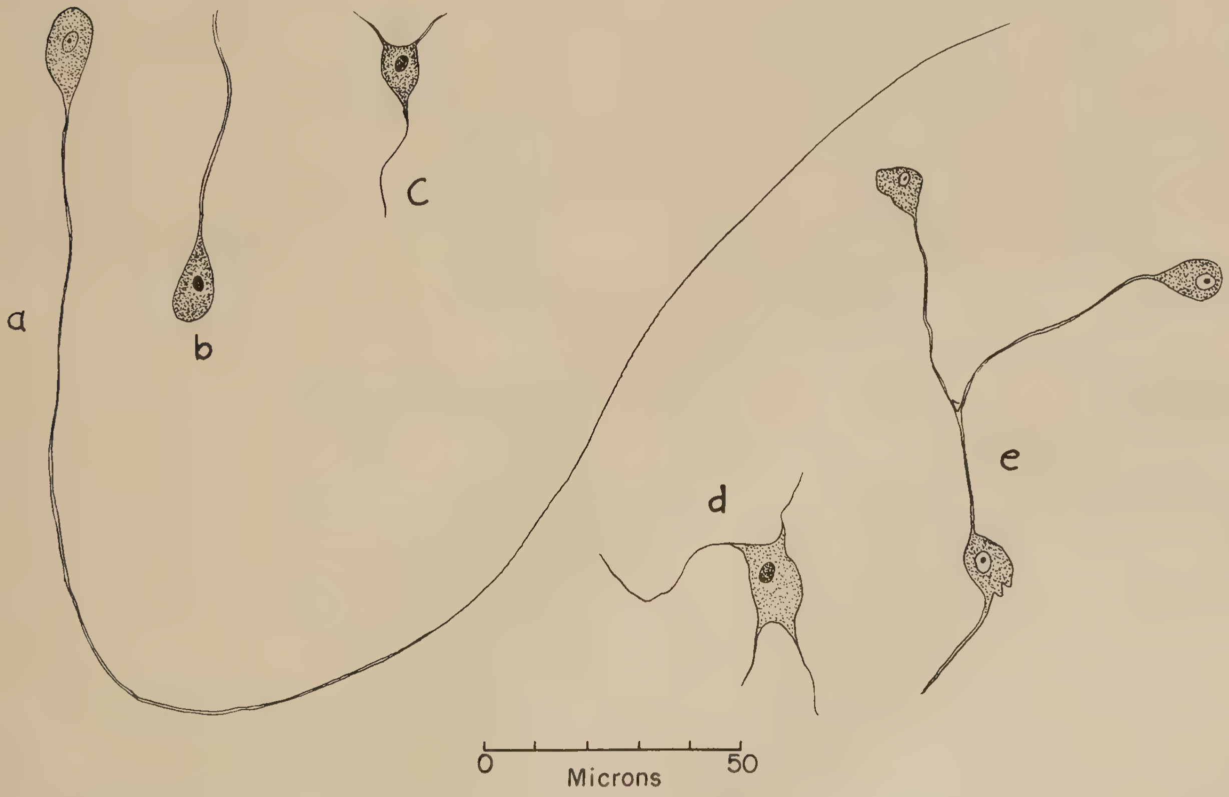
a
b
c
d
e
0
Microns
50

FIGURE 258.—Nerve cells isolated from the visceral ganglion of *O. edulis*, according to Rawitz (1887). a, b,—unipolar cells; c, d,—multipolar cells; e,—bipolar cells.

sectioned material of the oyster ganglia. The crossing of some fibers from the right to the left side and their extension into the connective can be seen, but there is no way to estimate the relative abundance of these fibers. There is not enough evidence to establish with certainty which fibers run from the nerve cells of the connective into the ganglion and which follow the opposite direction.

The tissues of the cerebral ganglion are less compact than those of the visceral ganglion, and the entire organ is rather indistinctly separated from the underlying connective tissue (fig. 259). The cerebral ganglia are located on each side of the oyster directly under the surface epithelium but separated from it by a thin layer of connective tissue fibers. The inner part of the cerebral ganglion (fig. 260) consists of bipolar cells of medium size. The outer layer, corresponding to cortex, is made up of large unipolar and multipolar nerve cells with dense protoplasm, stained dark with hematoxylin. Loose connective tissue covers the ganglion on the outer side. The entire organ is less compact than the visceral ganglion.

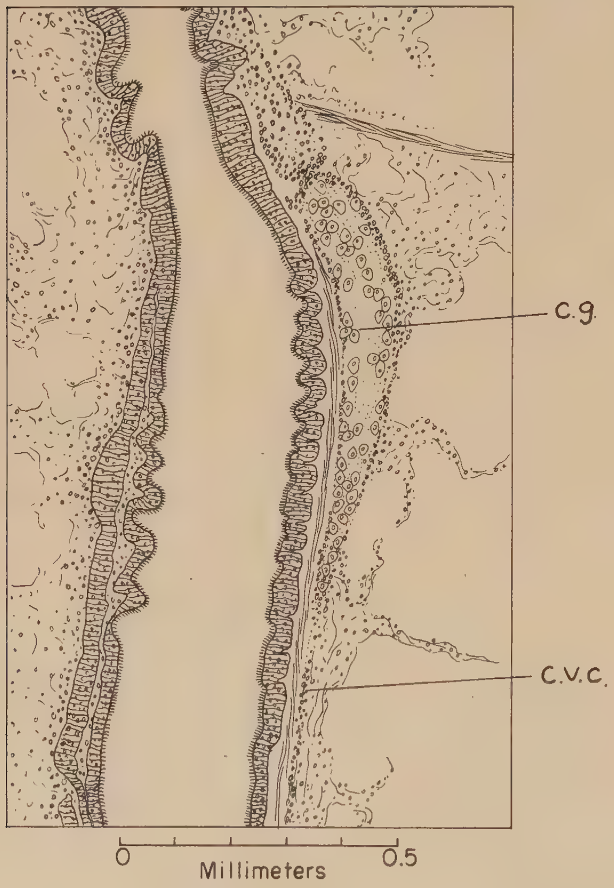

FIGURE 259.—Longitudinal section of a portion of the cerebral ganglion, (c.g.) at the base of the labial palps of *C. virginica;* c.v.c. the beginning of the cerebro-visceral connective. Kahle, hematoxylin-eosin.

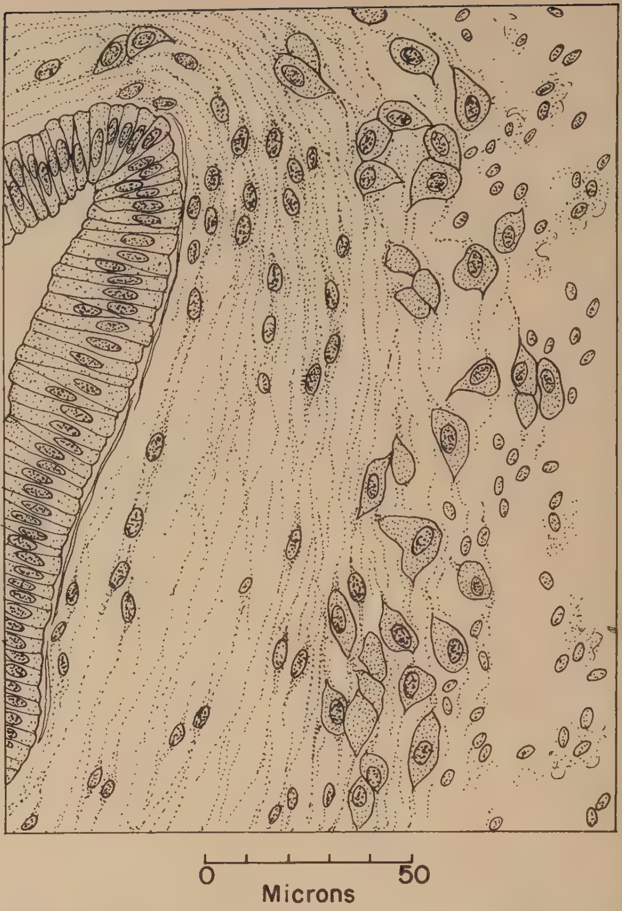

FIGURE 260.—Small portion of a longitudinal section of the cerebral ganglion of *C. virginica* under the epithelium of the base of the labial palps. Large unipolar and multipolar nerve cells form a cortex layer. Medium sized bipolar nerve cells and nerve bundles occupy the central part of the ganglion. Loose connective tissue covers the structure (right side of the drawing). Kahle, hematoxylin-eosin.

The structure of the cerebro-visceral connective is similar to that of the ganglia (fig. 261). A thick layer of large nerve cells surrounds the neuropile, which is divided into several bundles. There is no well formed sheathing, but small connective tissue cells are found along the periphery of the ganglion and are scattered throughout its entire structure.

A similar pattern of ganglionic structure repeats itself in many nerves emerging from the ganglia. This plan of organization is found in the circumpallial, branchial, and many other nerves.

The circumpallial nerve is surrounded by a sheath of connective tissue fibers (fig. 262). The

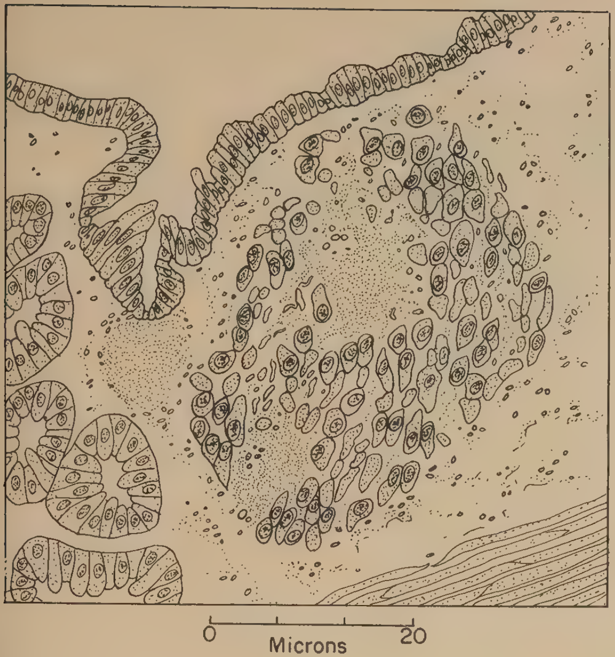

FIGURE 261.—Transverse section of the cerebro-visceral connective of *C. virginica*. Large aggregation of nerve cells on the periphery. Kidney tubules on extreme left. Kahle, hematoxylin-eosin.

nerve cells are predominantly at the periphery. Along its entire course the nerve gives rise to numerous branches which at regular intervals enter the tentacles and terminate at their surface in a number of small fibers which can be seen in gold-impregnated preparations (fig. 86, ch. V).

Small peripheral nerves such as the branchial nerves (fig. 263) and the radial nerves of the mantle (fig. 264) are made of bundles of fibers with occasional small nerve cells between them. The sheath of these nerves consists of a narrow layer of spindle-shaped connective tissue cells. A well-developed nerve net, visible in gold-impregnated sections (fig. 87, ch. V), is found over the entire mantle.

The anatomical and histological picture describes an intercommunication system which connects all the organs and parts of the oyster. Stimulus received, for instance, at the dorsoanterior part of the mantle and transmitted to the cerebral ganglia may reach the visceral ganglion either through the cerebro-visceral connective or directly via the numerous nerve branches which extend from the edge of the mantle and are connected by the circumpallial nerve. Stimuli transmitted to cephalic ganglia may reach the visceral ganglion through one of the connectives and vice versa. Thus, in spite of the absence of a central nervous system, the nervous reactions of the oyster are well integrated.

The major movements of the oyster are limited to the contraction and relaxation of the adductor muscle. Muscular contraction may be provoked either by the stimulation of the receptors of the tentacles and mantle or by impulses which originate from the internal organs. An example of the latter type of stimulation is found in the spawning reaction of the female. It consists of a series of rhythmic contractions of the adductor muscle associated with the release and dispersal of ova by the sexually mature oyster but in the immature specimen it cannot be induced by drugs, mechanical, electric, or thermal stimuli. The reaction is fully discussed in chapter VIII, page 172.

THE PALLIAL ORGAN

The only sense organs of the oyster are the tentacles of the mantle edge and the pallial (also called abdominal) organ. The structure and innervation of the tentacles have been already discussed in chapter V, page 85. The pallial organ is a very small structure attached to the anterior side of the adductor muscle inside the exhalant chamber of the gills. In order to see the organ the wall of the cloaca and of the epibranchial chamber must be dissected and the two sides drawn apart in the manner shown in figure 236 in chapter XI, page 259. The pallial organ is a small, colorless protuberance marked on the drawing by the letters pal.or.

The structure was discovered by Thiele (1889) in a number of bivalves iucluding *O. edulis*. In the European oyster the organ was described as a comma-shaped protuberance ("kommaformige Erhebung") with the pointed end turned to the right and the concave side oriented toward the posterior end. The organ on the right side is well developed but on the left side is small and degenerate. The description and illustration published by Thiele apply to *C. virginica*. In this species the pallial organ on the right side is also better developed while on the left side it is much smaller and frequently absent. A similar condition is found in *O. cucullata* (Awati and Rai, 1931).

The structure of the pallial organ of *C. virginica* is revealed in a series of sagittal sections (fig. 265). The rounded surface of the organ is covered by elongated epithelial cells with hairlike cilia which are longer than the cell bodies. This type of cell

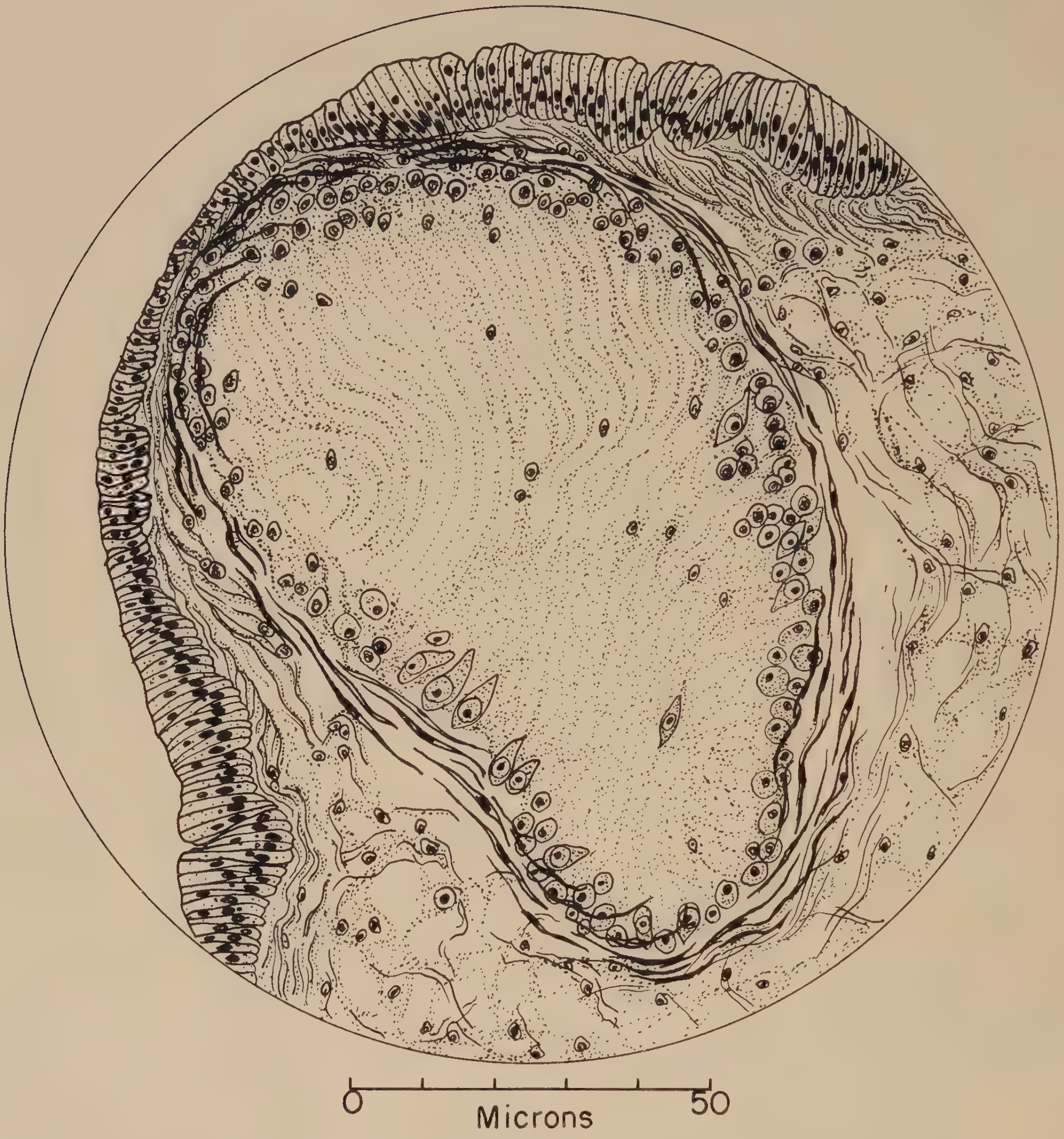

FIGURE 262.—Cross section of the circumpallial nerve of *C. virginica*. Kahle, hematoxylin-eosin.

closely resembles the so-called brush cells (Pinselzelle) described by Flemming (1884) in the sense organs of various mollusks. Thiele (1889) found such cells in the pallial organs of *Pinna, Avicula Arca, Lima,* and other bivalves. The epithelium of the pallial organ of *C. virginica* consists of two layers of cells: the deeper one is made of cells with globular nuclei, while in the surface layer the cells and their nuclei are oval-shaped (fig. 266). This observation is in agreement with the descriptions given by Thiele. The epithelial layer of the pallial organs of *Lima inflata* and *L. hians* is made, however, of a single layer (Studnitz, 1931). Thiele remarks that the cilia in *Arca noae* and *Lima* are motionless. It seems reasonable to assume that this may be true for the pallial organs of oysters. Unfortunately, the location of the pallial organ inside the exhalant chamber of the gills makes it impossible to study the function of the organ without inflicting serious injury to the surrounding tissues and nerves. From the histological picture it appears that stiff, hairlike cilia on the surface transfer the stimuli to the nerve endings and to the nerve trunk. Figure 266 represents a small section of the epithelial covering of the pallial organ of *C. virginica* examined at high magnification. Here the surface layer contains many sensory cells recognizable by their narrow

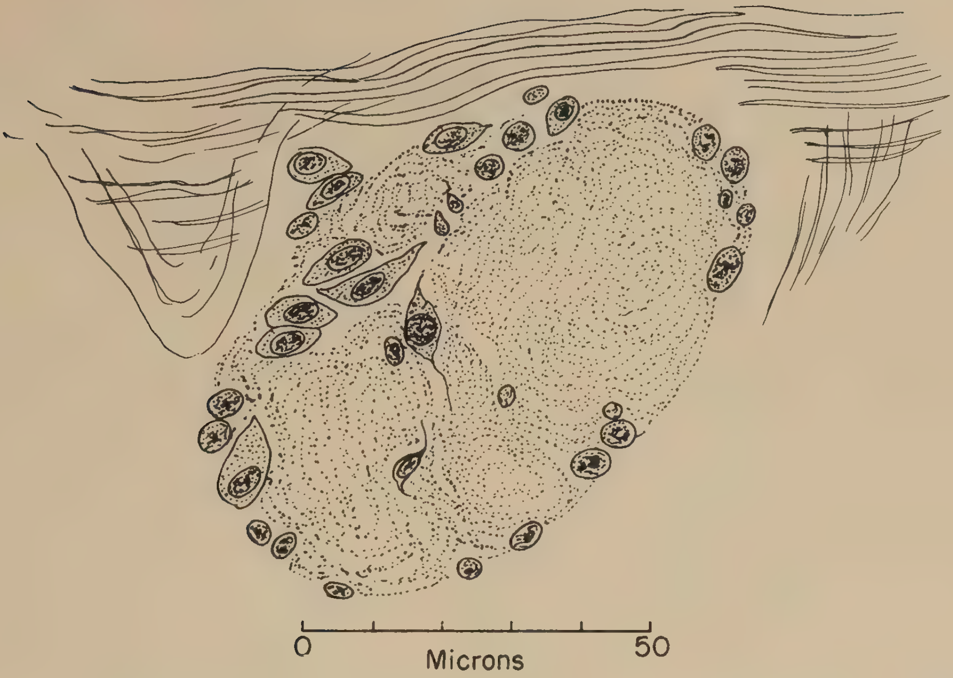

FIGURE 263.—Transverse section of branchial nerve of *C. virginica.* Gill muscles are above the nerve. Kahle, hematoxylin-eosin.

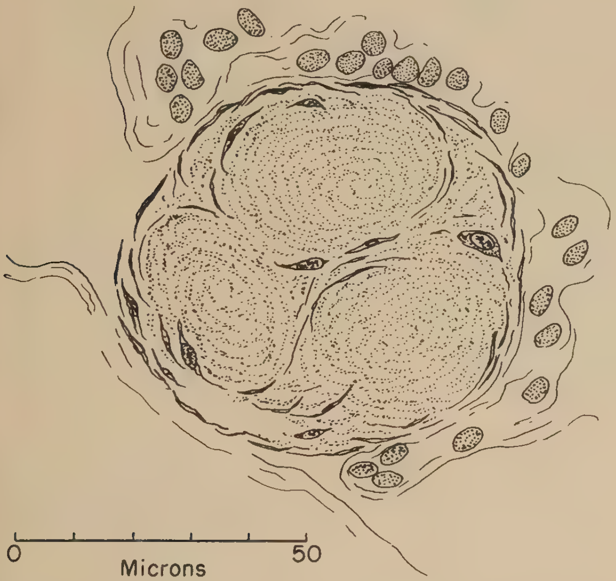

FIGURE 264.—Radial nerve in the mantle of *C. virginica.* The sheathing of connective tissue forms the periphery. Here the nerve consists of three distinct nerve trunks. Blood cells are scattered in the connective tissue of the mantle. Kahle, hematoxylin-eosin.

bodies and long processes which extend from the surface of the organ deep into the underlying connective tissues. The nuclei are elongated, and the cells are deeply stained with iron and alum hematoxylin. A large nerve trunk, shown in figure 265, sends its branches to the surface of the pallial organ. The nerve entering the organ is a branch of one of the posterior pallial nerves which emerge from the visceral ganglion. Typical vesicular connective tissue forms the core of the organ.

There has been no experimental study of the pallial organ, and its physiology is not well understood. Elsey (1935) expressed the opinion that its primary function is the regulation of respiratory current. Haas (1935) thought that in *Lima* the pallial organ is primarily concerned with chemical testing of water, and this view is repeated by Franc (1960) in his review of Bivalvia.

The long, stiff cilia seem to be more suitable for detecting mechanical disturbances than for responding to chemical stimuli. The organs of chemical taste in the oyster are the tentacles of the mantle. Because of their location at the edge of the mantle they are the first ones to come in contact with the irritating or poisonous substances in water and give the oyster a signal to prevent its access into the pallial cavity. It appears unreasonable that an organ of chemical taste should be located inside the water transporting system near its end. Dahmen (1923) and Awati and Rai (1931) think that the pallial organ in bivalves is primarily the organ for detection of

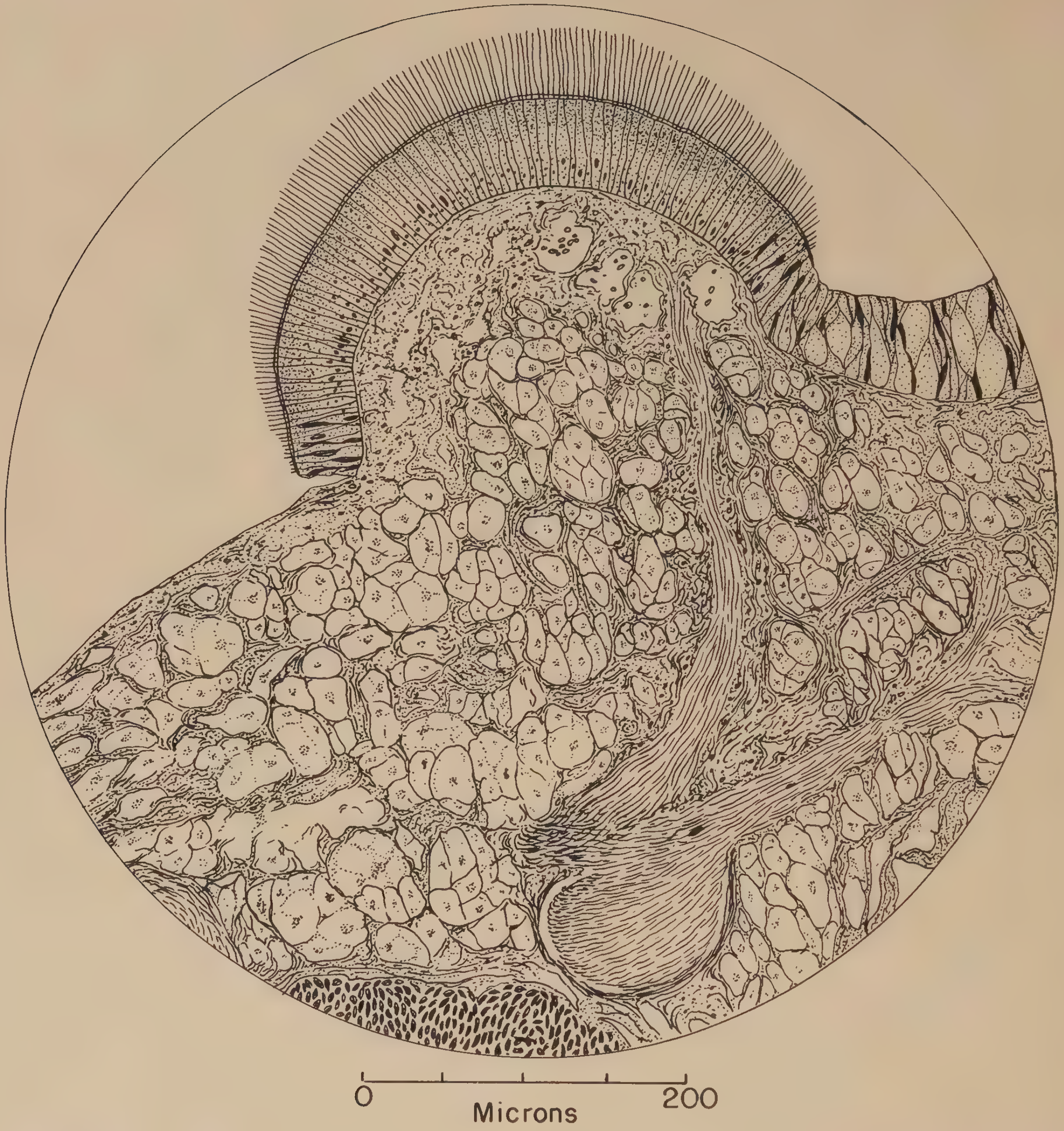

FIGURE 265.—Sagittal section of the pallial organ of *C. virginica*. Long ciliated cells rest on semiglobular protuberance of connective tissue richly supplied with blood vessels and ramifications of the posterior pallial nerve. Kahle, hematoxylin-eosin.

mechanical disturbance in the surrounding water. A final answer to the enigma of the pallial organ must await results of physiological studies which so far have not been undertaken.

A different type of pallial sense organ of the spat of *O. edulis* was described by Cole (1938), who found a thin-walled spherical and pigmented sac projecting from the inside mantle surface near its border, about one-third of the length of the free edge from the mouth. The cavity of the sac contains a comparatively large noncalcareous and nonsiliceous concretion which stains visibly with eosin. There are no cilia inside the sac, which is covered by very attenuated epithelium. Cole remarks that the organ is suitably constructed and in a favorable position

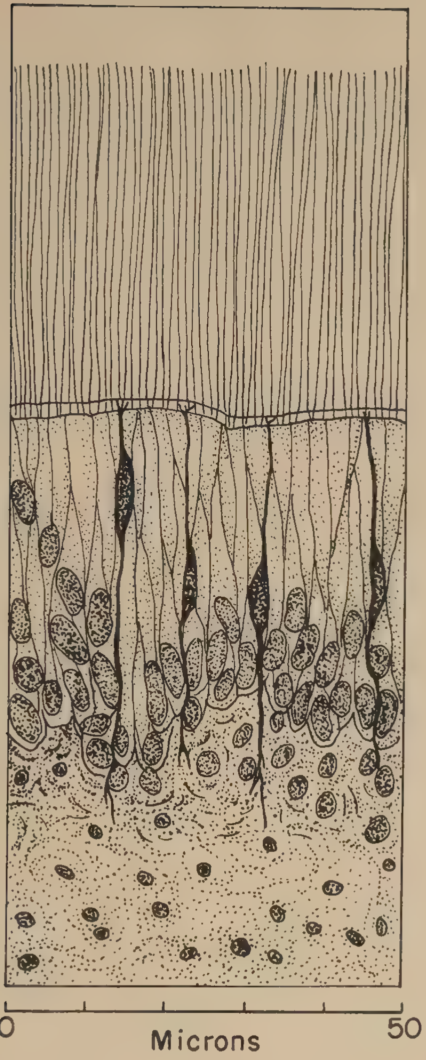

FIGURE 266.—Section of a small part of the ciliated covering of the pallial organ of *C. virginica* showing sensory cells between the ciliated epithelial cells. Kahle, iron hematoxylin.

for receiving and reacting to vibration. He found that this organ is fully developed in 4- to 5-day-old spat. It is not known whether the organ is present in adult *O. edulis* or in *C. virginica*.

SENSORY STIMULATION

Very little experimental work has been conducted on the physiology of the nervous system of the oyster. Most of the research on neurophysiology of other bivalves (*Pecten*, *Mytilus*, *Mya*, and *Anodonta*) dealt with the action potentials, tonus of the adductor muscle (see: ch. VIII), and stimulation of the siphons of *Mya* (Hecht, 1919a, 1919b, 1920a, 1920b; Piéron, 1941) and *Pholas* (Hecht, 1928). From a study of the action potentials along the nerve trunks of the siphon of the soft shell clam, Piéron calculated that the velocity of the transmission of stimuli along the nerve of this mollusk is of a magnitude of several meters per second. The value is probably common to other bivalves.

Neuro-secretory cells are present in a number of marine bivalves (*Nucula*, *Anomia*, *Mytilus*, *Modiolus*, *Chlamys*, *Lima*, *Donax*, *Arcopagia*, *Mactra*, *Cardium*, *Venerupis*, *Venus*, and *Paulora*) and probably may be found in other genera including the oyster (Gabe, 1955). These cells are typical small neurones of the cerebral and visceral ganglia but are absent in the pedal ganglia (Lubet, 1955). The amount of secretion they contain varies with the season and is apparently related to or parallels the sexual cycle for it increases with the maturation of sexual products (see: ch. XIV, page 312).

Sensory stimulation of the tentacles of the oyster by chemicals was studied by Hopkins (1932a, 1932b). He measured their sensitivity by determining with a stopwatch the latent period, i.e., the time elapsed between the application of a chemical and retraction of a tentacle or a group of them. The method is very simple. The mantle is exposed by cutting off a portion of shell, and the oyster is placed in sea water running at a constant speed through a rectangular tank with two communicating parts, one of them shallow and the other several inches deeper. Water in the taller part is kept at a constant level. The oyster is placed in the shallow portion of the tank, and a vessel containing the solution to be tested floats in the taller part. A three-way stopcock is mounted on the wall separating the two parts of the tank, one branch of the stopcock is bent horizontally and ends in a capillary nozzle placed a short distance in front of the tentacles. The two other branches are fitted with flexible rubber tubing at the end; one is lowered into sea water, the other into the floating vessel with the test solution. At the beginning of the test the stopcock is turned to deliver a constant, gentle stream of sea water to the tentacles, which remain in a relaxed state as long as the current and temperature of the water are constant. The stopcock is turned abruptly, and the sea water is suddenly replaced by the solution to be tested. The total time from the turning of the stopcock to the observed retraction of the tentacles is measured with the accuracy of one-tenth of a second. Before making a test the time required for a test solution to pass from the container to the tip of the nozzle is recorded by using a colored solution and the value obtained is subtracted from the total time measured, giving the duration of the actual latent period. Since the vessel in the deeper part remains afloat, the level from which the solution is withdrawn remains constant and consequently there is no change in the velocity of current striking the tentacles.

All parts of this simple apparatus may be built of plastic. The following precautions should be taken: 1) the nozzle in front of the oyster must be firmly mounted and placed on a solid stand to avoid mechanical disturbance when turning the stopcock; 2) water and test solutions should be kept at equal and constant temperature; and 3) levels from which the sea water or the test solution are delivered to the tentacles should be constant in order to avoid a change in the velocity of current.

This method was satisfactorily used in the Woods Hole laboratory in testing the reaction of oysters to various organic compounds and contaminants.

Sensory stimulation of tentacles by inorganic salt solutions depends on the chemical composition of the substance used and its concentration. The relationship between the concentration of a given substance and the latent period, presented in Hopkins' papers, indicates that the effect, considered as the reciprocal of the latent period, is directly proportional to concentration. Sometimes a secretion of mucus covers the tentacles and impedes the reaction. In such cases Hopkins found it necessary to subtract a constant from the latent period values.

The range of latent period values in Hopkins' experiments varied from a fraction of a second to about 15 seconds. By using 0.5 M solutions of several inorganic salts Hopkins arrived at the conclusion that sensory stimulation is primarily the function of cations, which he listed in the following order of effectiveness: $\mathrm{K} > \mathrm{Na} > \mathrm{NH_4} > \mathrm{Li}$.

Tentacles respond also to chemical stimulation by various organic compounds such as quinine sulphate, cumarin, etc. An odorous compound such as cumarin is detected by the oyster in a concentration of 0.0004 percent. The oyster responds also in a measurable reaction to a 0.004 percent solution of quinine sulfate. This concentration is one-eighth of the strength of the solution of quinine that can be detected by man's tongue (Hecht, 1918).

Cane sugar has little stimulating effect on the tentacles (Hopkins, 1932b). Tests with fructose (in sea water) that I made in the Woods Hole laboratory gave the following results:

0.05 percent	No reaction in 1 minute.
1.0 percent	Latent period 8 to 12 seconds in four out of 10 trials.
5.0 percent	Latent period 4 to 8.4 seconds.

Arabinose is more effective:

50 mg./l	Latent period 26 to 49 seconds in five out of 10 trials.
100 mg./l	Latent period 12 to 34 seconds in all 10 trials.
5.0 percent	Latent period 5 to 8 seconds in all 10 trials.

An interesting reaction was observed when diluted sea water (one part fresh water + three parts sea water) was used. The inner lobe of the mantle curled up, but the tentacles were not affected. The gradual curling ended in a sharp contraction of the adductor muscle. Normal sea water added to the edge of the nantle in a control experiment produced no such result.

A similar protective reaction was observed when the extracts of the meat of the oyster drill (*Urosalpinx cinerea*) and of the stomach of the starfish (*Asterias forbesi*) were used. The tentacles reacted strongly in contact with the undiluted juice of *Urosalpinx* with the latent periods of 1.5 to 3.6 seconds. These experiments suggest that sensory mechanism of the oyster may be sufficient to detect the close proximity of carnivorous gastropods.

The mechanism of sensory stimulation has not been adequately studied and is not fully understood. Its biological significance is, however, apparent. The warning received by the tentacles is transmitted through the circumpallial nerve of the mantle to the ganglia. If stimulation is sufficiently intense either a part or the entire mantle is withdrawn, the entrance to the gills is closed, and the ensuing contraction of the adductor firmly closes the shell. The three steps outlined can be observed under experimental conditions; they constitute the three distinct phases of the defense reaction of the organism.

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CHAPTER XIV

ORGANS OF REPRODUCTION

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ANATOMY

The ripe gonad is a massive organ located near the surface of the body within a layer of connective tissue between the digestive diverticula on the inner side and the surface epithelium on the other. In sexually mature oysters it appears as many branching tubules (follicles) which merge along the dorsal side of the body to form one continuous structure encompassing the visceral mass and extending ventrally to the tip of the pyloric process (fig. 267).

The reproductive gland is not encapsulated, and its outlines are indistinct. At sexual maturity the surface covering of the body becomes so thin that a network of fine genital canals is clearly visible through it. The diameters of the canals gradually increase as they converge into a wider gonoduct through which the germ cells are discharged. Two separate systems of genital canals, one on each side of the body, are the only sign of the paired origin of the sex gland which in an adult oyster is completely fused into a single organ.

In many bivalves sex products are discharged through the kidneys, but in the oyster the gonoduct opens into a vestibule, or atrium, which also receives the urinal duct. This relationship can be seen on a series of slightly slanted sections of the lower part of a ripe gonad. One of these sections is shown in fig. 268. The female oyster from which the tissue was taken was preserved during the act of spawning. The follicles of the lower part of the ovary (left side of the figure) are almost empty of ripe eggs; a short ciliated passage between the ovarian follicle and the pear-shaped area of the kidney vestibule are at the right; two spawned eggs are near the outside opening of the vestibule, which is lined with ciliated epithelium. A transverse section of the kidney reservoir lined with typical secretory cells is in the upper right portion of the figure. The connection between the vestibule and the reservoir is located above this section. The follicles in the inner portion of a gonad lie at an angle to the genital ducts into which they discharge their content. The structure of spermary differs from that of the ovary only in that the follicles are filled with spermatozoa.

The degree of sexual development can be estimated roughly by measuring with calipers the thickness of a transverse section of the gonad layer. Since the gonad is not uniformly thick in all its parts, the section should be made at some selected place. In conformity with the practice used by the Biological Laboratories of the Bureau of Commercial Fisheries at Milford, Conn., Oxford, Md., and Gulf Breeze, Fla., the oyster is cut with a razor blade along a line extending from the lower corner of the labial palps across the stomach to the posterior end of the body. There is considerable variability in the gonad layer of oysters of known age and similar environment. In Long Island Sound the average maximum thickness of ripe gonads of 4- to 5-year-old oysters taken from three depth levels of 10, 20, and 30 feet was, according to Loosanoff and Engle (1942), about 2.4 mm. for the shallow water oysters and only about 1.5 mm. for those found in deeper water. Much greater gonadal development was recorded in other locations. Some fully mature Cape Cod oysters used in my experimental work had a layer of gonad from 6 to 8 mm. thick, and a similar degree of development was noted in oysters from a small tidal pool near the laboratory at Beaufort, N.C.

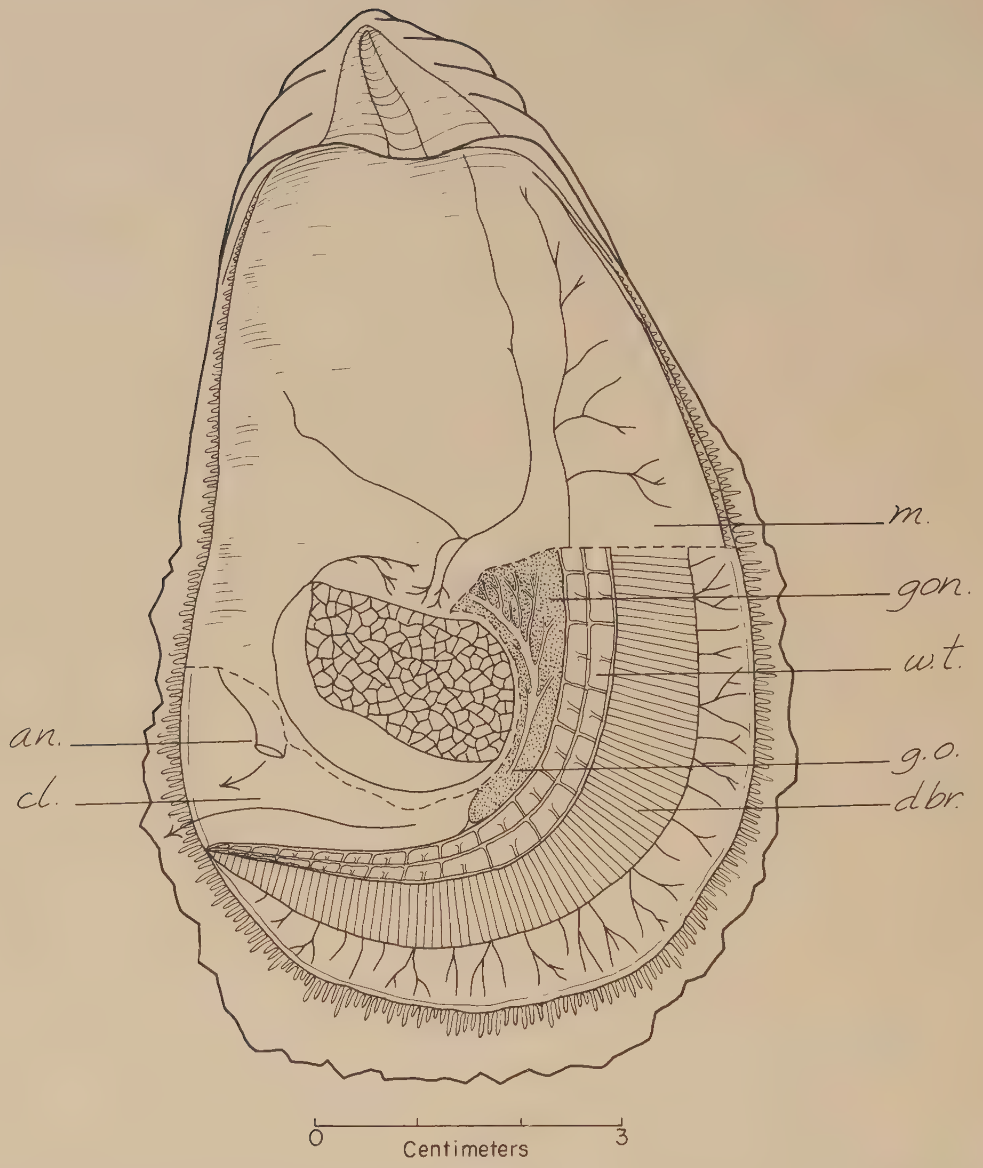


FIGURE 267.—Fully ripe *C. virginica.* The mantle of the right side was dissected above the epibranchial chamber and pushed to the right to expose the pyloric process. A network of canals leads to the single gonoduct through which the sex cells are discharged. Drawn from life. an.—anus; cl.—cloaca; d.br.—right demibranch; g.o.—genital opening; gon.—gonad; m.—mantle; w.t.—water tubes inside the gills.

Gonadal layers of about 10 mm. thickness were found in large *C. gigas* (8 inches in height) from Willapa Bay, Wash. (Galtsoff, 1930a, 1932).

In all species of *Crassostrea* the bulk of gonads varies from season to season, reaching its maximum shortly before the onset of spawning. The number of sex cells produced during one reproductive period varies, depending on the conditions of the environment. The greatest gonadal development is more likely to be found in the populations of oysters from the northern latitudes north of the Chesapeake Bay rather than in the south Atlantic and Gulf waters. This is apparently associated with the fact that the reproductive season in the northern latitudes is of short duration, 4 to 6 weeks, while in the warmer waters of the south gonadal formation and spawning, continue, with interruptions, for several months. In both groups the annual reproductive capacity (i.e., the number of eggs produced annually) maybe of the same order of magnitude or even greater in the southern oysters because of the longer reproductive period, but the greatest bulk of a ripe gonad is more likely to occur in oysters which have only one spawning period per year.

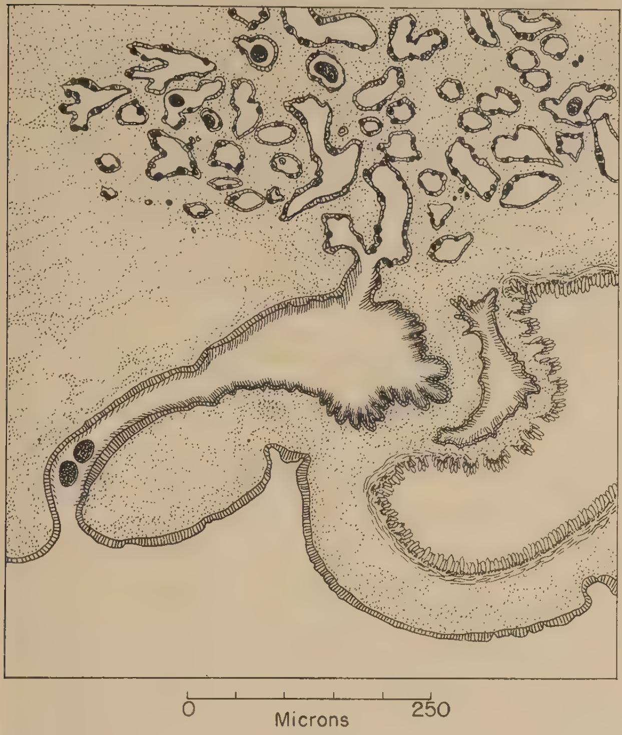


FIGURE 268.—Slightly slanted tangential section through the opening and adjacent portion of the ovary of *C. virginica*. Oviduct with two eggs at the lower right side. Kidney reservoir at upper right. Ovary follicles at left. Drawn semidiagrammatically from a photomicrograph of a preparation. Kahle, hematoxylin-eosin.

Several factors besides geography influence gonadal development. The most significant are temperature, depth (Loosanoff and Engle, 1942), salinity, available food, and pollution of oyster bottoms. Many examples of the suppression of gonadal growth by adverse conditions may be cited. For example, oysters living in waters highly polluted by various trade wastes have, as a rule, poorly developed gonads. Sometimes the development of the sex gland is suppressed to such a degree that only traces of follicles are found in the visceral mass, and the layer of the digestive diverticula is visible from the surface. These oysters have the greenish or brownish coloration typical for the digestive diverticula which in the sexually ripe oyster is not noticeable under a thick layer of gonad. Oysters with suppressed gonad development are found in the waters which receive continuous discharge of the pollutants from pulp and paper mills. Similar poor oysters are frequently encountered in waters of extremely low salinity or from areas where salinity increases to the highest limit of tolerance (34 to 40 ‰). The determination of gonadal thickness described above lacks precision because variation in the compactness of the gonadal tissue cannot be measured, but the method is, nevertheless, useful for the practical purpose of estimating expected intensity of spawning.

DETERMINATION OF VOLUME AND WEIGHT OF GONAD

The fully developed gonad is the largest organ of the oyster. The ovaries or spermaries can be separated from the underlying digestive diverticula by using small curved scissors. The excised pieces are weighed, and their volume is measured by displacement in a simple device made from a glass cylinder of appropriate dimensions (depending on the size of the sample) with a drain pipe at the bottom and a side glass tubing of about 5 mm. in diameter fused to the side at an angle of about 45° to record the water level. A convenient water level is selected and recorded, the tissue is introduced, and the water is then drained into a measuring vessel to the previous level. The body weights of adult New England oysters with fully developed gonads varied in my observations from 14.2 to 23.2 g. The gonads comprised from 31.2 to 40.7 percent of the total body weight exclusive of shell. The volume of the oysters' tissues ranged from 21 to 24 ml., with the gonads accounting for 32.8 to 33.4 percent of the total bulk. Oysters selected for these measurements were of the highest commercial quality and with maximum development of gonads. In poor oysters with light gonadal development the proportion of the weight of the gonad to body weight is only a small fraction of the figures given above.

HISTOLOGY

The gonads of the oyster originate from a group of primordial germ cells located in the mesodermal band on the ventral side of the pericardium in the vicinity of the visceral ganglion (Coe, 1943a). In embryos of bivalves primordial germ cells are identified by their relatively large size, round shape, and clear vesicular nucleus with one or two nucleoli (Okada, 1936, 1939; Woods, 1931, 1932). The primordium soon becomes separated into two groups which by continuous multiplication of the constituent cells extend symmetrically along both sides of the body. Each group grows anteriorly, surrounded by vesicular connective tissue of the visceral mass, and forms a system of profusely branching tubular follicles. The fusion of the branches along the dorsal side obliterates any remnants of the paired origin of the gonad.

The microscopical structure of the gonad varies, depending on the age of the oyster, degree of maturity, season, and environmental condition. In Ostreidae and in some other pelecypods (*Pecten*, *Mytilus*, *Volsella*) the follicles of a fully developed gonad consist almost entirely of primitive sex cells (gonia) at various stages of development with only a few minute follicular cells between them. Because of the absence of a capsule or membrane around the gonad, the sex cells are in direct contact with the surrounding tissues (fig. 269). It may be assumed that the role of follicular cells in the growth of the gonad of oysters is insignificant and that the gametogenic cells obtain their nourishment directly from the connective tissue which surrounds them. In *Mya*, *Teredo*, *Bankia*, and other Adesmacea the follicular cells are large and function as accessory nutritive cells.

In immature oysters and in the adults, that had completed the spawning period, the germinal epithelium consists of undifferentiated sex cells, some at the early stages of gametogenesis. Their sex can be recognized only by careful cytological examination. As the follicles grow and ramify they spread throughout the surrounding layer of connective tissue. The maturing sex cells inside them multiply, grow, and fill up the lumen (fig. 270). The follicles near the surface of the gonad are distinctly different. Their outer walls facing the body surface are lined with ciliated epithelium, and only the inner sides of the follicles retain the germinal cells (fig. 271). This differentiation of the germinal epithelium into two distinct types is probably common to all species of oysters. The follicles lined with ciliated cells, function as the genital canals through which mature sex cells are moved by ciliary action. They were first described by Hoek (1883) for *O. edulis* and subsequently were found in *O. lurida* and in several species of *Crassostrea*.

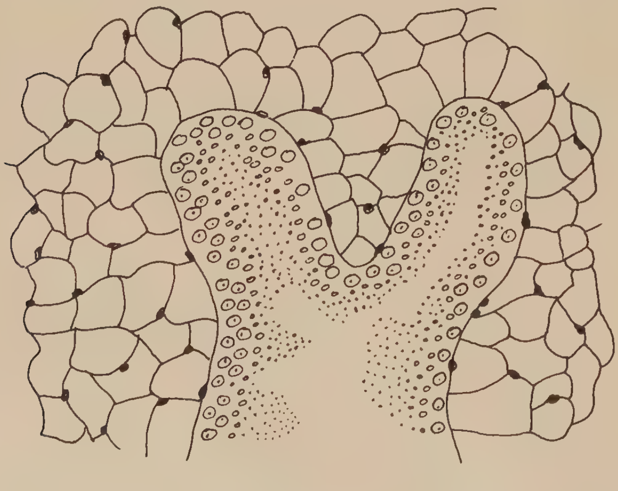


FIGURE 269.—Terminal portion of the follicle of a gonad of *C. virginica* at early stage of development (male phase). The follicle is surrounded by a large mass of connective tissue. Redrawn from Coe, 1936a.

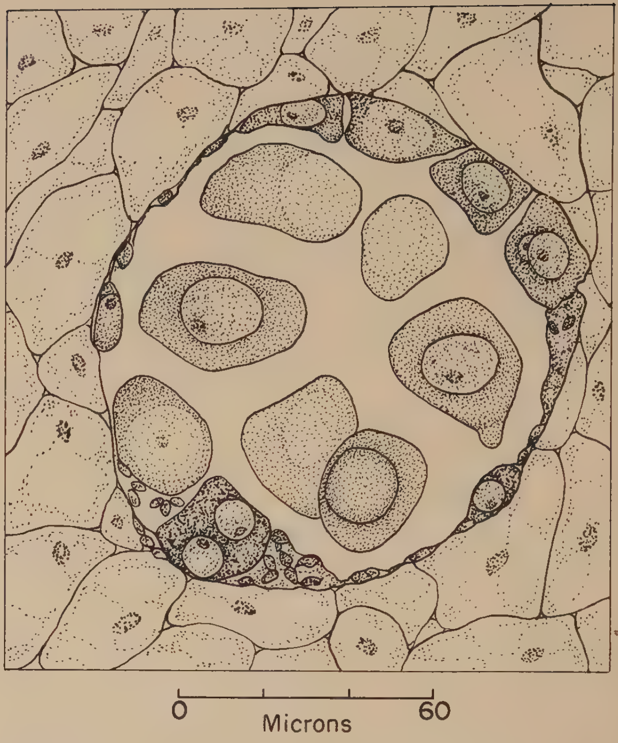


FIGURE 270.—Cross section of one surface follicle in a fully developed ovary of *C. virginica*. Bouin, hematoxylin-eosin.

Since the transformation of germinal cells into ova and sperm is a gradual process which does not involve all the cells of the germinal epithelium at the same time, numerous undifferentiated or so-called residual cells are usually found along the inner periphery of a follicle. Some of them can be found even in a fully developed gonad (fig. 270).

The bulk of a functional ovary is made up of fully developed ova which fill up the lumina of

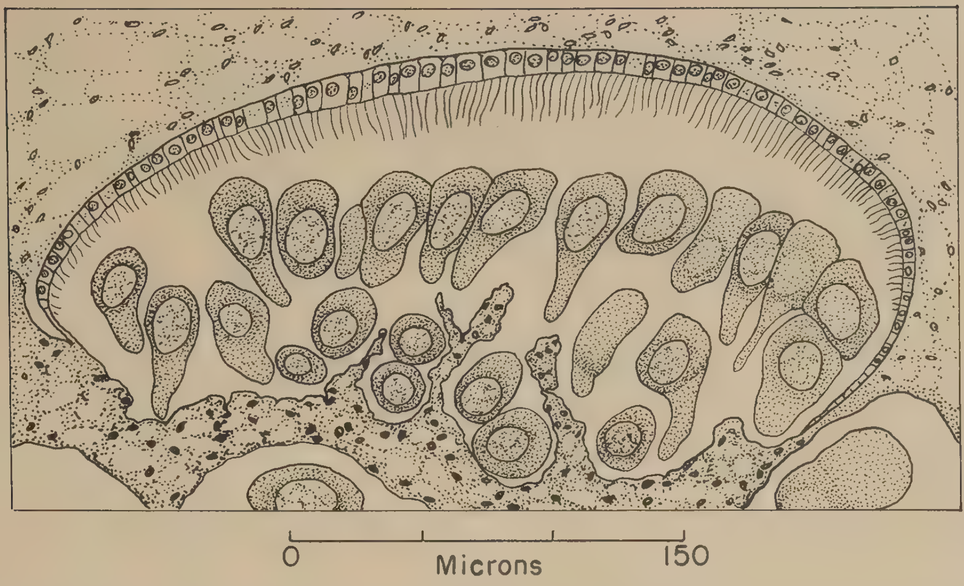


FIGURE 271.—Cross section of a surface follicle from a fully developed ovary of *C. virginica*. Germinal epithelium is formed by cells at different stages of ovogenesis and by small undifferentiated cells. Bouin, hematoxylin-eosin.

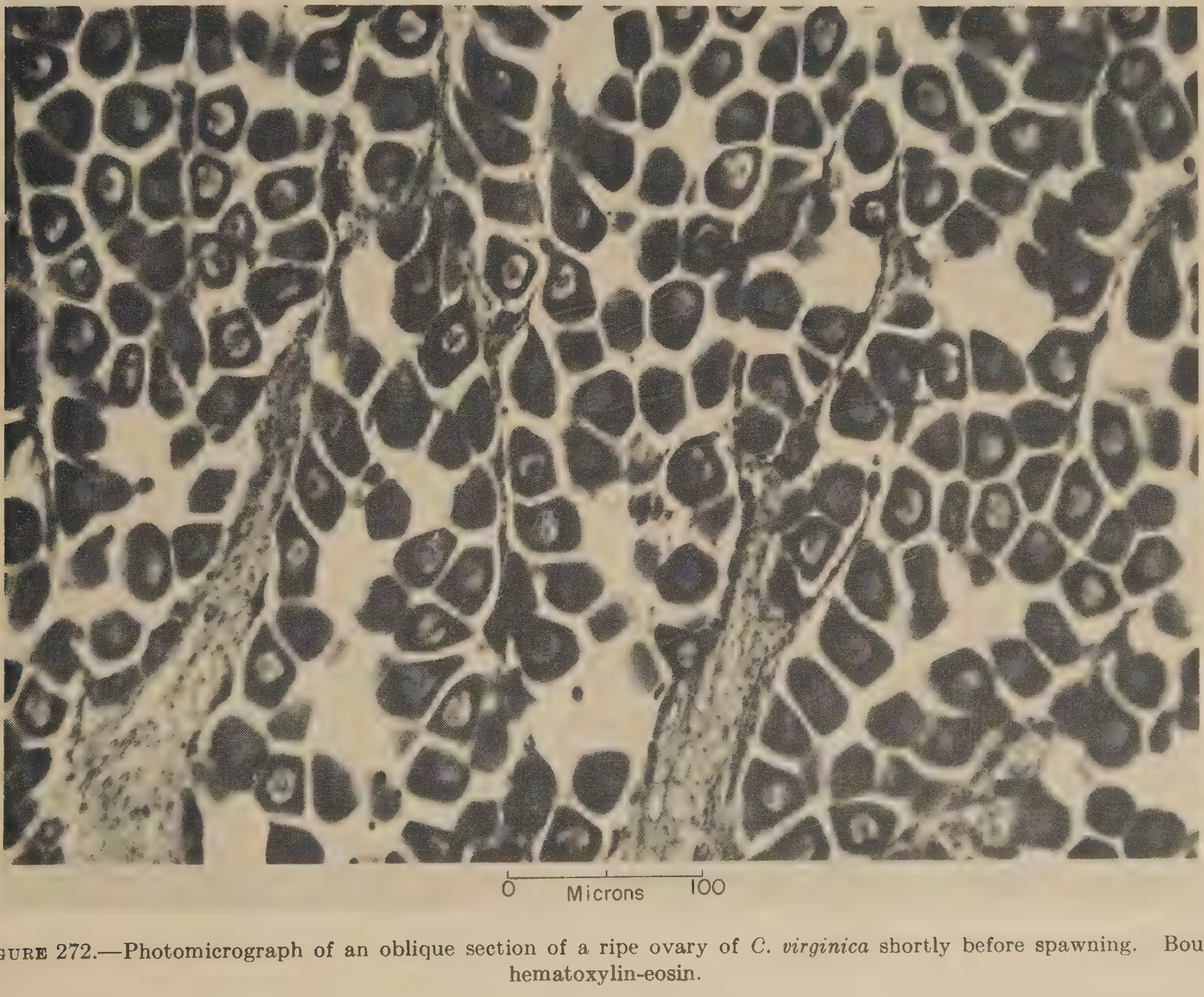


FIGURE 272.—Photomicrograph of an oblique section of a ripe ovary of *C. virginica* shortly before spawning. Bouin, hematoxylin-eosin.

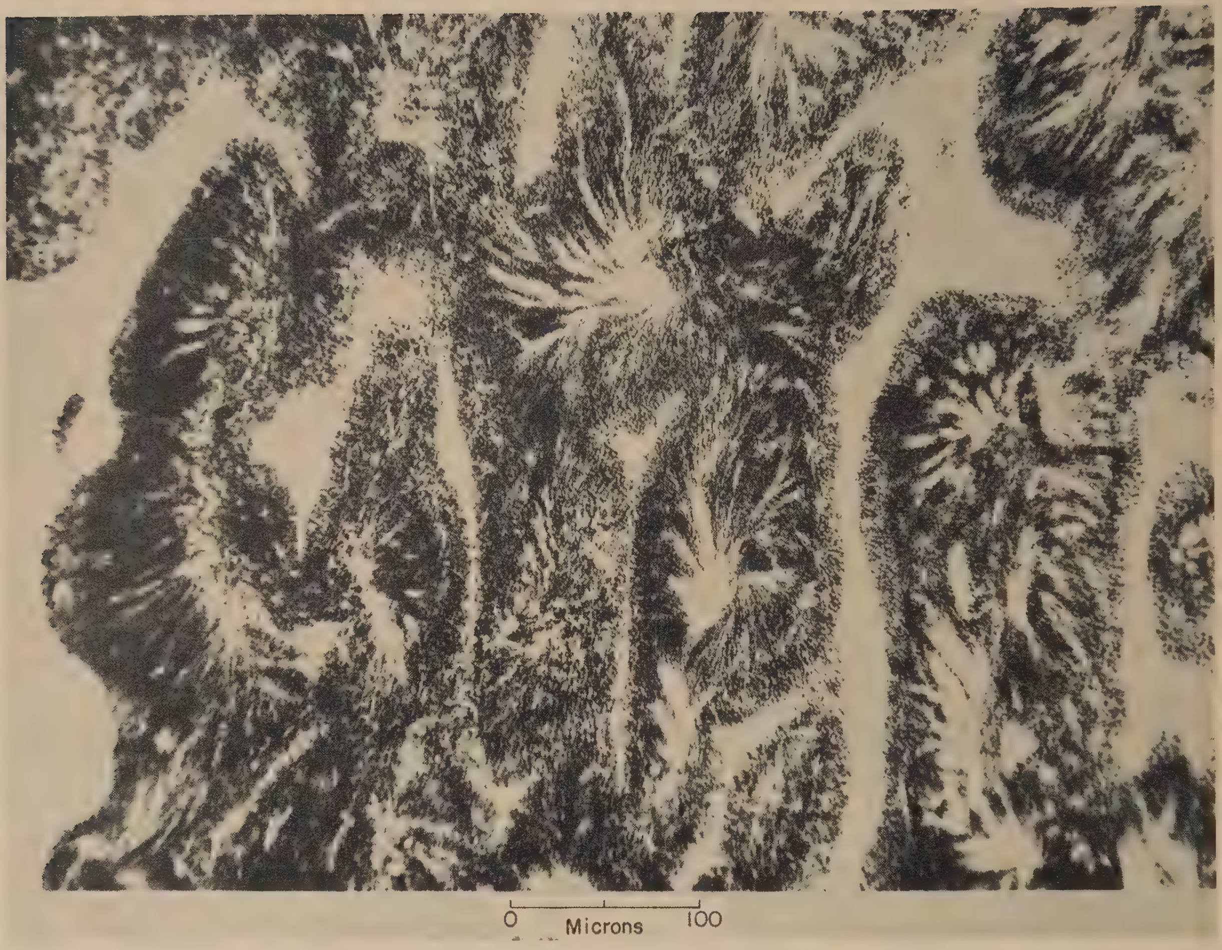


Figure 273.—Photomicrograph of a cross section of a ripe spermary of *C. virginica.* Bouin, hematoxylin-eosin.

the follicles and of a number of cells at different stages of the development nearer to the walls. The eggs are attached to the walls of the follicles by elongated peduncles, giving them a pear-shaped appearance. At the height of sexual development the layer of undifferentiated germinal epithelium is reduced to a very narrow band of small cells hardly visible at low magnification (fig. 272). The connective tissue between the follicles also very nearly disappears.

The arrangement of cells in a ripe spermary (fig. 273) is similar to that in the ovary. Fully formed spermatozoa are massed together inside the follicle with their tails inward. They become separated as the sperm moves through the genital canals, which are frequently distended by the accumulation of spermatozoa ready to be ejected. In *O. edulis* and *O. lurida* the sperm in the lumen of a follicle form distinct balls which retain their shape until they are discharged by the oyster. A number of sex cells remain in the follicles at the completion of spawning. Consequently, at the end of the reproductive season the gonads contain mature ova and sperm as well as undifferentiated cells; many of them are pycnotic and detached from the germinal lining of the wall. Phagocytosis becomes pronounced as large numbers of leucocytes invade the follicles to digest and cytolize the remaining sex cells (fig. 274). The connective tissue between the follicles becomes disorganized. After the reabsorption of the gonad is completed only a narrow band of germinal epithelium remains in a few follicles and the entire layer has shrunk to such a thin band that it is not visible to the naked eye. The oyster is now at an indifferent stage. Its sex can be recognized in some individuals in which young ovocytes or spermatocytes are present, but in many others

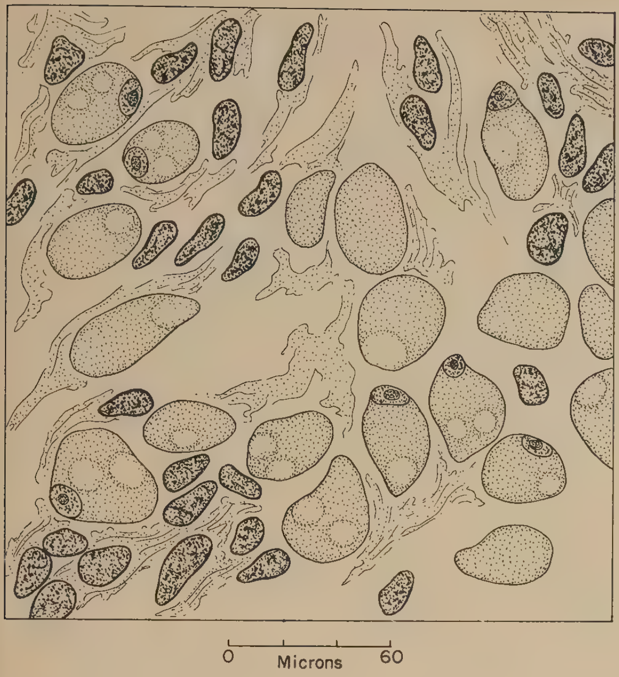


FIGURE 274.—Ovary of *C. virginica* shortly after completion of spawning. Unspawned ova are cytolized. Note the invading phagocytes and the disorganization of connective tissue. Bouin, hematoxylin-eosin.

the young sex cells are not sufficiently differentiated and their sexes can not be identified. Ovocytes, when sufficiently developed, can be distinguished from spermatocytes by their large nuclei and granular cytoplasm.

During spawning the sex cells are moved inside the genital canals by ciliary motion of the epithelium. How they are released from the follicles and reach the genital canals located near the surface of the sex gland is not known. Histological examination discloses no contractile elements in the tissues surrounding the follicles, and no contraction of the gonad or part of it could be detected during spawning. Upon reaching the gonoduct the released cells continue to be moved by the powerful cilia of the duct and vestibule (fig. 275). Longitudinal and circular muscle fibers are found under the basal membrane of the vestibule and appear to be better developed in the male gonad than in the female. Roughley (1933) refers to the presence of sphincter muscles in the gonoducts of *Ostrea* (*Crassostrea*) *commercialis*, but no structure resembling a sphincter is found in *C. virginica* (Galtsoff, 1938a). It is conceivable that a contraction of circular muscle fibers of the spermiduct to a certain degree controls the passage of sperm through the genital opening, but no such action has been detected under experimental conditions.

The epithelium of the distal end of the gonoduct and of the urinogenital vestibule contains, besides the ciliated cells, a large number of mucous cells (MC) not present in the lining of the canals. In the ovary the opening of the oviduct into the vestibule is marked by a small ridge of ciliated cells (Galtsoff, 1938a, 1938b).

SPAWNING

The sexual apparatus of oysters is of the simplest type for it lacks the accessory sex organs which in some mollusks are used for mutual excitation, storage of sex cells, copulation, and secretion of egg capsules. In spite of the structural simplicity of the reproductive organs, the spawning of the female oyster is a rather complex action which involves coordination of the gills, nervous system, mantle, and the adductor muscle, while the sexual act of the male is much simpler. It is, therefore, more convenient to describe separately the phenomena involved in the spawning of the two sexes. Under natural conditions simultaneous release of sperm and eggs, essential for successful reproduction of the species, is attained through mutual stimulation.

SPAWNING REACTIONS OF THE FEMALE

Spawning of the female proceeds in several consecutive steps which, in the order of their participation, involve the ovary, the gills, the mantle, and the adductor muscle. The behavior of these organs follows a distinct pattern, one action succeeding the other in a precise sequence which finally terminates in the dispersal of eggs in the surrounding water.

The first step is ovulation, i.e., the discharge of eggs from the ovary into the epibranchial chamber. The moment the eggs appear in the epibranchial chamber the two edges of the mantle come together and effectively seal the pallial cavity and cloaca. This peculiar behavior of the mantle may be observed in a spawning female oyster placed close to the wall of a rectangular tank. In an actively feeding animal the space between the two mantle edges is wide open; the pallium and the tentacles are extended outward parallel to the surface of the valves and the gills, exposing the side of the adductor muscle, the rectum, and the inner part of the cloaca (fig. 276). A different picture is seen in a spawning female. A few minutes before ovulation the edges of the

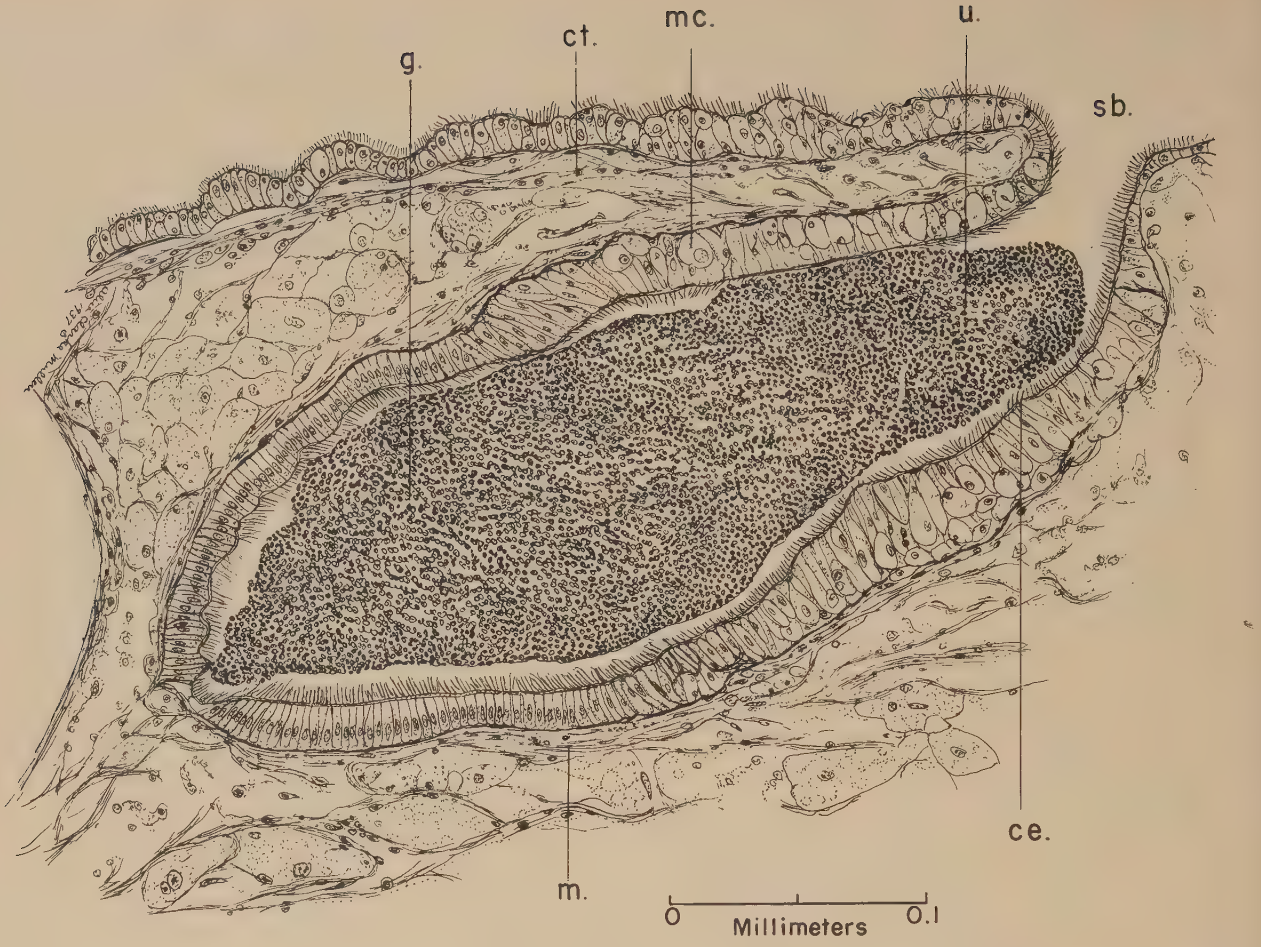


FIGURE 275.—Section through the lowest part of spermiduct and the adjacent portion of the vestibule. Both organs are grossly distended by the discharged sperm. Tissue of *C. virginica* was preserved during the act of spawning. G—approximate position of the junction of spermiduct and the vestibule; CE—ciliated epithelium; CT—connective tissue; M—longitudinal muscle fibers; MC—mucous cell; SB—opening of the vestibule into the epibranchial chamber; U—lower part of the urinogenital vestibule. Bouin, hematoxylin-eosin. From Galtsoff, 1932.

mantle display unusual activity; they come together and temporarily close the access to the gills; then for a few seconds they open again. Both the rate and range of shell movements at this time gradually increase. Finally, the entrance to the gill cavity closes completely except for one small opening or "window" as shown in fig. 277. Soon a white cloud of unfertilized eggs appears at the window, the adductor muscle contracts sharply, and the eggs are discharged and dispersed several inches away from the oyster (fig. 278). The opening between the edges of the mantles may be formed at any place along their periphery but once formed its position remains unchanged throughout the duration of spawning. Spawning may last from a few minutes to nearly 1 hour, depending on the amount of mature eggs in the ovary.

Eggs trapped inside the epibranchial chamber have to pass through the water tubes in order to accumulate in the space between the gills and the mantle since there is no other way by which they can reach this area (Galtsoff, 1938a). This conclusion was confirmed by microscopic examination of a section of the gills of a female preserved during the act of spawning (fig. 279). Any other minute particles suspended in water pass through the ostia and water tubes into the exhalant chambers and are swept by the outgoing current. The eggs released through the genital pore, however, take the opposite course when they enter the water tubes of the gills.

While the eggs pass through the gills the ostia are wide open and the ciliary currents along the filaments are neither inhibited nor reversed. The eggs, therefore, flow against the current produced

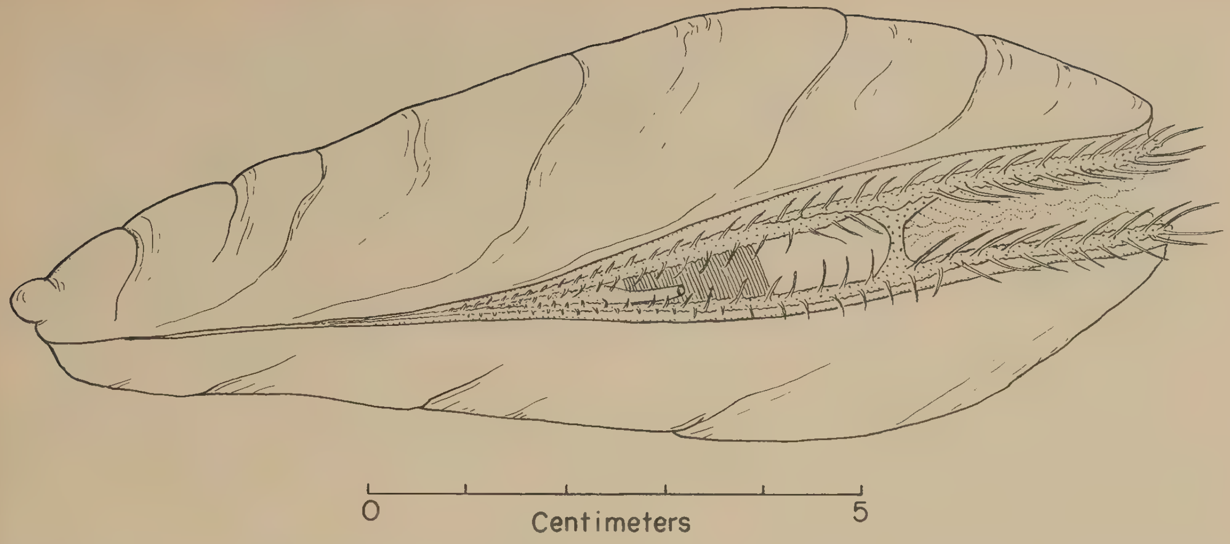


FIGURE 276.—Actively feeding *C. virginica*. The valves are wide open, the pallium and the tentacles are extended outward and the cloaca, the adductor muscle, and rectum are clearly visible. Drawn from life. The opening between the valves is slightly exaggerated.

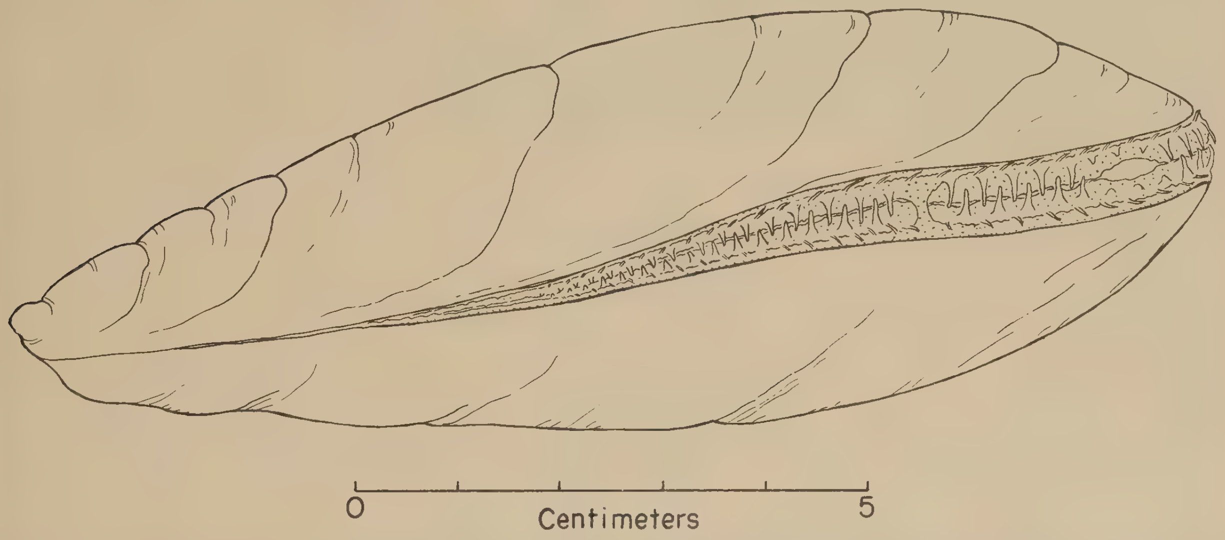


FIGURE 277.—Position of the mantle and tentacles of *C. virginica* shortly before the discharge of eggs through a small window left open (right side of figure). Drawn from life.

by the lateral cilia. Laboratory observations show that when the valves open the gill lamellae also expand and the water tubes dilate. At the same time the closing of the cloaca and of the mantle cavity cuts off the access of water from the outside. As a result, a suction is produced inside the water tubes by the expansion of gill lamellae, forcing the eggs into the water tubes and through the ostia to the surface of the gills.

Eggs of larviparous oysters (*O. lurida* and *O. edulis*) also pass through the gills but are retained in the pallial cavity until fully developed larvae are formed and escape from the mother's body.

Stafford (1913) thought that the eggs of *O. lurida* are too heavy to be carried by the respiratory current and so fall into the water tubes and are forced through the ostia by the pressure of their own mass. The correctness of such an explanation seems doubtful. The act of spawning in the larviparous species has not been studied adequately, probably because the ovulation and passage of eggs into the pallial chamber proceed without any outward indication (Yonge, 1960). Eggs of these species are fertilized inside the body

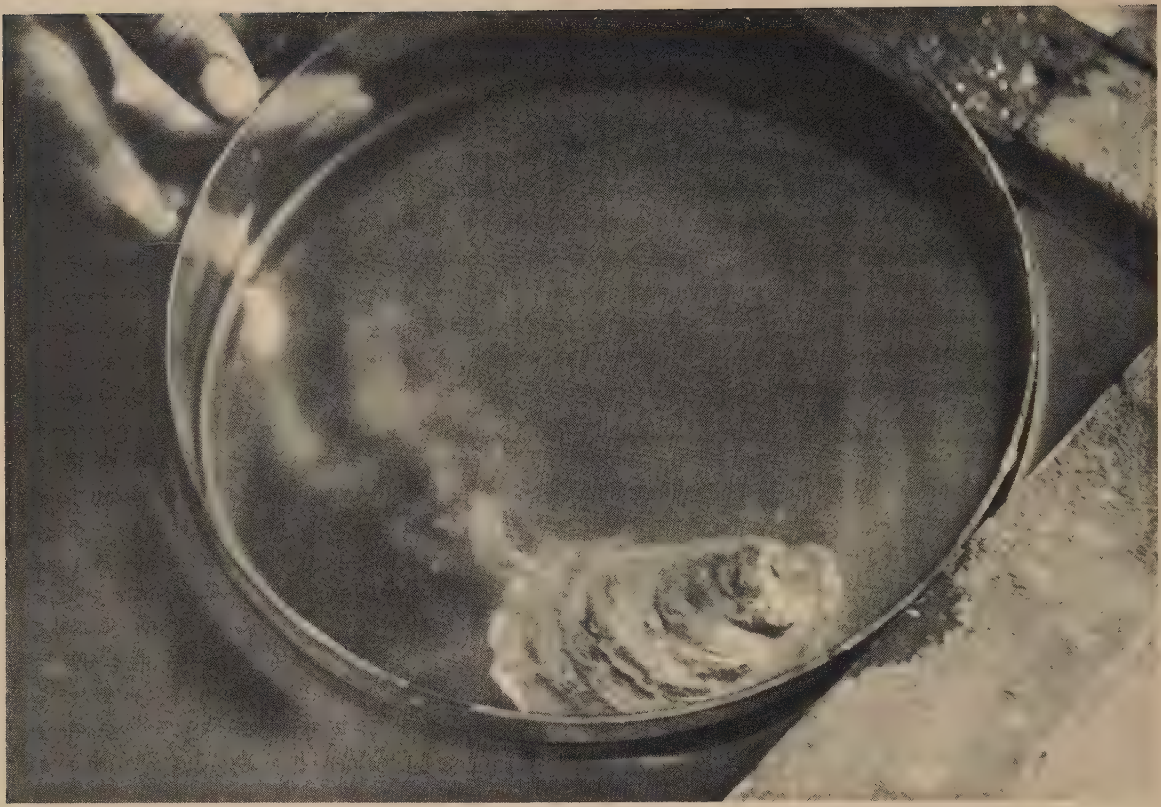


FIGURE 278.—Spawning of large female *C. virginica*, photographed in the laboratory.

by sperm drawn in from the outside with the respiratory current and are extruded as well-developed larvae. The process is called "swarming" (Korringa, 1941). Careful studies of shell movements of *O. lurida* or *O. edulis* during the reproductive period may uncover some peculiarities of the behavior of their adductor muscles associated with swarming.

If the shell movements of a spawning female are prevented by cutting off a piece of valve between the adductor muscle and the hinge, the eggs cannot pass through the gills and are discharged through the cloaca. This has been demonstrated in the experiments illustrated in fig. 280, A and B. In both cases fully mature Cape Cod oysters were placed in finger bowls under a low-power binocular microscope. In oyster A the gills were exposed by cutting off a piece of the right valve without injuring the adductor muscle. Its shell movement remained normal. In oyster B the entire dorsal half of the right valve above the muscle attachment was removed, and in this way shell movements were prevented. During the spawning of oyster A the released eggs (e) passed through the gills, while in oyster B they were discharged through the cloaca.

Female spawning of *C. gigas* and *O. cucullata* follows the same pattern as the American oyster (Galtsoff, 1932). It is apparent that the mantle, gills, and adductor muscle of *Crassostrea* species temporarily assume the role of accessory sex organs and through coordination and adjustment of their activities perform a specific role which is distinct from their primary functions.

The release of sex cells from sexually mature oysters often requires a stimulus which causes a triggerlike effect and initiates spawning. Very often this effect is associated with a sudden rise in the temperature of the water. Numerous ecological observations show that under natural conditions oysters spawn at rising temperature. This led to the concept of "critical temperature," and for many years the temperature of 20° C. was considered the lowest at which spawning takes place. It was postulated that "once this critical temperature' of 20° C. is reached a trigger mechanism is released which requires some hours for its consummation" (Nelson, 1928a). Further observations disproved this concept. Nelson reported that *C. virginica* transplanted from the United States to England could be induced to spawn at 19.1° C. (Nelson, 1931). Some of the oysters of Long Island Sound spawn at 16.4° C. (Loosanoff, 1939).

Ecological evidence shows that spawning of an oyster population often coincides with a rapid rise of temperature but it is not determined by a specific "critical" temperature. Physiological studies at the Woods Hole laboratory indicate that temperature and chemical stimulation, acting singly or jointly, may induce spawning in sexually ripe oysters. On the other hand it is apparent that certain internal and external conditions inhibit spawning.

The effect of temperature on spawning can be observed by placing a sexually mature oyster in a tank of water, connecting its right valve to the writing lever of a kymograph, rapidly warming the water and then maintaining the temperature at a desired level. Shell movements of the

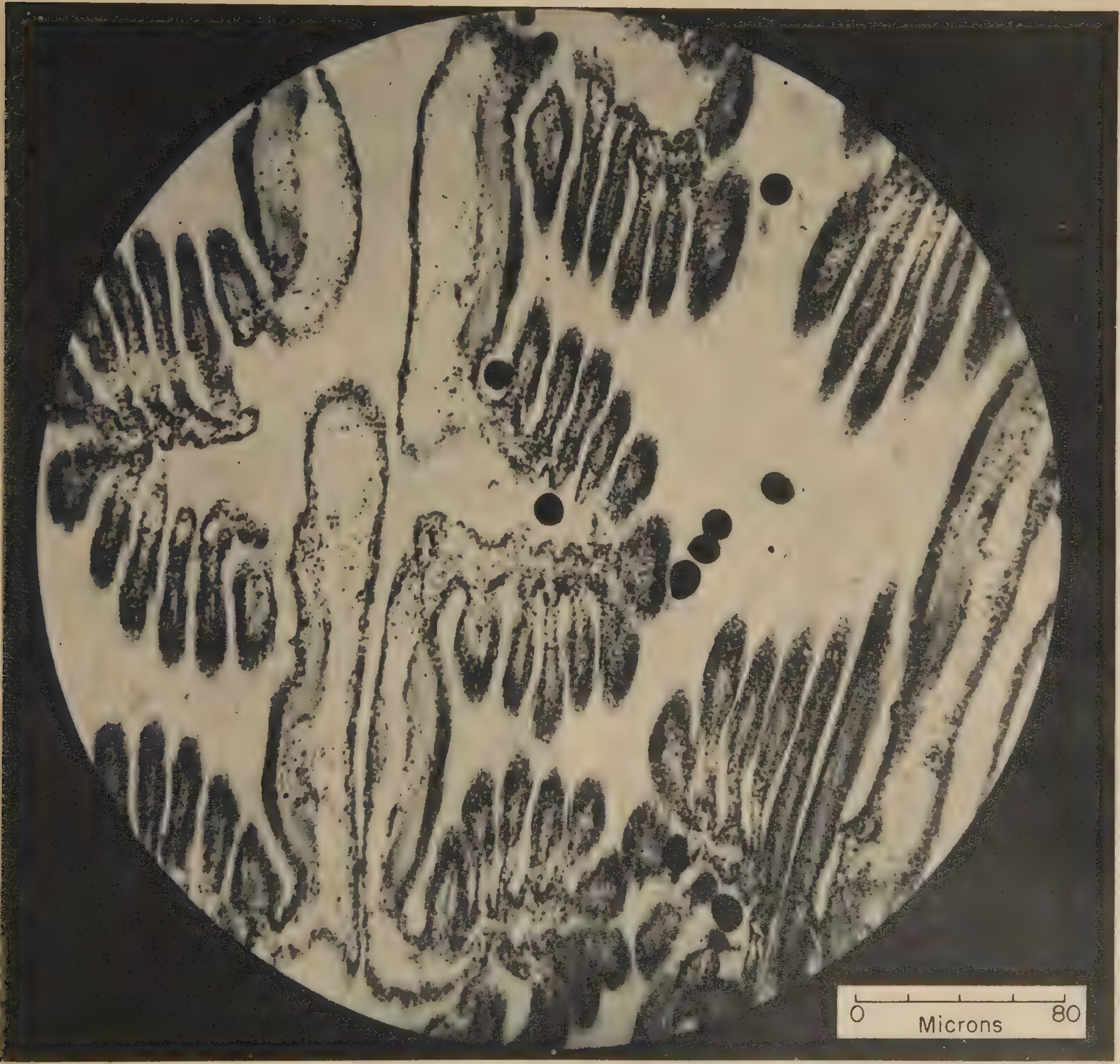


FIGURE 279.—Transverse section of the gills of female *C. virginica* preserved during spawning. Hematin-eosin. Note the eggs inside the water tube (center) and in the ostium.

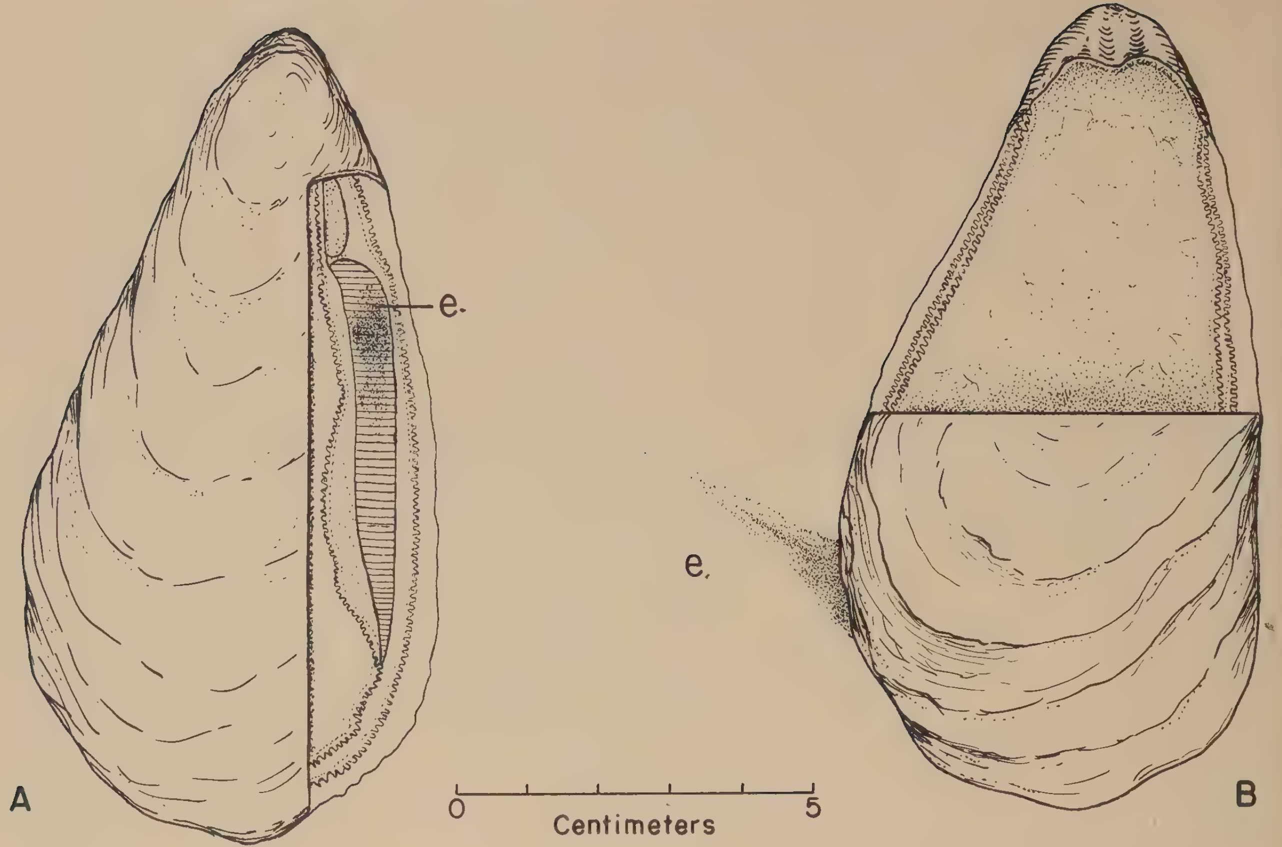


FIGURE 280.—Experiment showing the role of shell movements in the discharge of spawned eggs of *C. virginica* through the gills. A.—Portion of the right valve was removed to expose the gills; the adductor muscle was not injured and shell movements during spawning were normal. Eggs (e) pass through the gills. B.—Portion of the right valve between the adductor muscle and the hinge was cut off to prevent shell movements. Eggs (e) pass through the cloaca. Drawn from life.

spawning oyster are recorded on a kymograph, and the presence of discharged eggs or sperm in the water is ascertained by microscopic examination of samples taken at frequent intervals. In the case of heavy spawning so many sex cells may be shed that the water becomes milky and opaque; when there is light spawning the presence of eggs should be checked by collecting material which settles on the bottom of the tanks.

Spawning of sexually ripe females of *C. virginica* may be induced by warming the water from 18° to 20° C. to 22° to 23° C. and maintaining this temperature for several hours. Relatively few oysters respond to this mild stimulation. A more effective method, which in my experience gave positive results in about 40 percent of the oysters tested, consisted in rapidly raising the temperature of the water from about 20° C. to 33° to 34° C. The remaining 60 percent of the oysters which did not respond to thermic stimulus required additional stimulation by live sperm. Using this technique I found that the population of oysters from a single small bed in Onset, tested within a few days, consisted of individuals which greatly varied in the degree of their response to spawning stimuli. The tests were made at intervals of 2° C. The females that failed to spawn at 22° to 23° C. spawned at this temperature when sperm was added to the water. Some of the oysters spawned at 25° to 27° C., but still others required the addition of sperm to induce ovulation at this temperature level. Similar results were obtained at 29° to 31° C. and 32° to 33° C. In each of the groups tested there were specimens which did not respond to the rise of temperature and required additional stimulation by live sperm. All the oysters used in these experiments were mature; they had fully developed gonads, the eggs were fertilizable, and spawning, when induced, was copious.

The threshold temperature of spawning is not a "critical" temperature in the sense that it automatically induces the discharge of eggs in all physiologically ripe oysters. The success or failure of thermic stimulation depends on the responsiveness of the organism. It would be more appropriate to speak of the "critical condition" of the organism which makes it responsive to stimulation rather than of critical temperature of spawning. Within broad limits between 15° and 32° to 34° C., spawning of *C. virginica* may occur at any temperature; mass spawning of an oyster population is more likely to take place in warm water above the 22° to 23° C. level.

Stimulation by live sperm is of great importance in the reproduction of *Crassostrea* oysters. In the Woods Hole experiments the time elapsed between the addition of sperm suspension and the beginning of shedding of eggs varied between 6 and 38 minutes. At about 20° C. the sperm added to the pallial cavity passed through the gills and was expelled from the cloaca within 7 to 8 seconds. The latent period of spawning reaction lasts several minutes. This suggests that possibly the sperm acts upon the female organism after it has been absorbed by the cells of the water transport system or by the digestive tract. Direct evidence, however, is absent since attempts to prevent the penetration of sperm into the digestive tract by plugging the mouth and esophagus were not successful.

Rhythmic contractions of the adductor muscle are associated with the release of eggs from the ovary and are not directly stimulated by temperature or by any known chemical agent. This becomes clear from the observations which show that spawning contractions proceed in the same manner whether the spawning was induced by temperature or by sperm. Two kymograph tracings of shell movements of the two females shown in fig. 281 are similar in spite of the fact that in one of them (upper line) spawning was induced by the addition of sperm, while in the other by rapidly warming the water from 21.6° to 30.2° C.

Experiments were made to determine whether some substances causing the contraction of the adductor muscle are released into the blood stream during spawning. A female was induced to spawn by thermic stimulation, and a sample of its blood withdrawn from the pericardium was immediately injected into the visceral mass and into the circulatory system of a sexually mature but nonspawning female. Six experiments of this type were made with negative results.

Shell movements during female spawning are so typical that they cannot be mistaken from any other type of muscular activity. Spawning reaction is recognized by the duration of the latent period of not less than several minutes; the uniformity of the tonus level at the points of relaxation; regular rhythm of the contractions, particularly at the beginning of the reaction; and the presence of a small "plateau" about half-way on the relaxation curve (see fig. 281). The plateau is indicative of the slowing down of the relaxation phase; its significance is due to the fact that it coincides with the oozing out of eggs through the ostia of gill filaments (Galtsoff, 1938b). This type of

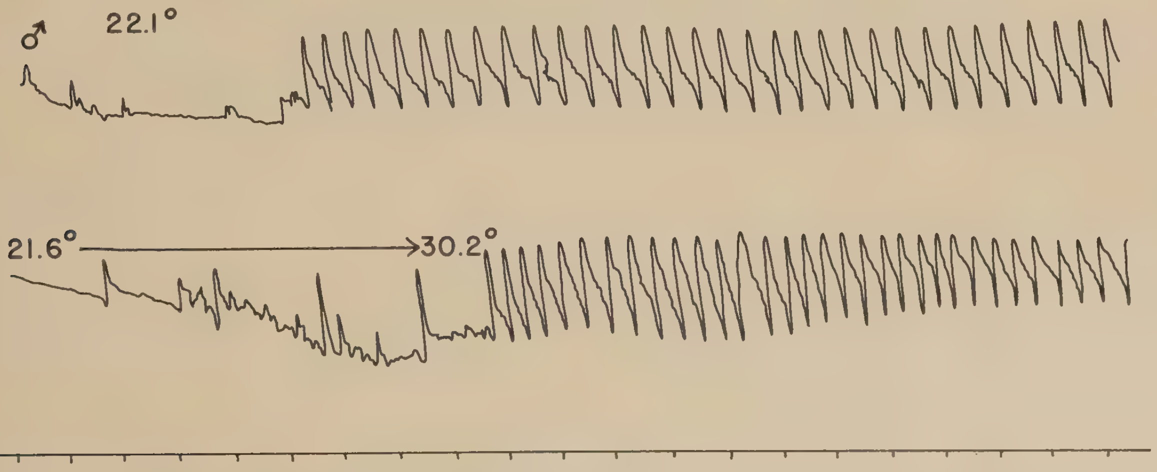


Figure 281.—Kymograph records of shell movements during spawning of two female *C. virginica*. Upper line—spawning induced by the addition of sperm at 22.1° C.; lower line—spawning induced by rapid rise of water temperature from 26.1° to 30.2° C. Time interval, 1 minute.

spawning curve appears in hundreds of records obtained in the laboratory in the course of several years of studies. It does not occur after the spawning season is over and cannot be provoked by temperature or chemical stimulation of the oysters devoid of mature eggs. Injections of low concentrations of adrenalin cause rhythmical contractions of the adductor muscle but of an entirely different type. The spawning reaction is always followed by a refractory period of two to several days during which the female is not responsive to stimulation.

SPAWNING REACTION OF THE MALE

Spawning of the male does not involve the participation of the mantle and adductor muscle. Sperm is discharged from the spermary into the epibranchial chamber by ciliary motion inside the genital ducts and is swept away by the respiratory current (figs. 282 and 283). The pallium remains wide open and quiescent. Muscular contractions of the adductor play no role in the release and discharge of sperm, and there is no visible change in the velocity of the cloacal current during ejaculation (Galtsoff, 1938a). Shedding of sperm occurs sometimes in sudden outbursts of brief duration which may be repeated at frequent intervals. Toward the end of the reproductive season the discharge of sperm may continue for several hours without interruption until the male is completely spent. Ejaculation proceeds either from one or from both spermiducts simultaneously. In the latter case the flow of milky water containing suspended spermatozoa can be seen emerging from the cloaca and from the promyal chamber simultaneously. The males of *C. gigas and C. commercialis* behave in a manner similar to the males of *C. virginica.*

Males of *C. virginica* are more responsive to spawning stimuli than the females of the species. They are more readily stimulated by rising temperature, and shedding of sperm is easily induced by various substances; a suspension of eggs or filtered egg water (sea water in which eggs were kept for some time); eggs of various bivalves (*Pecten irradians, Mercenaria mercenaria, Mytilus edulis*); and eggs of starfish, *Asterias forbesi.*

The latent period of stimulation varies depending on the substance used and its concentration, but in general it is much shorter than in female spawning. Suspension of eggs or egg water of *C. virginica* induces spawning of the male within 5 to 6 seconds at 24° to 25° C.; eggs of *Pecten irradians* are more effective, provoking a response in a male oyster in 4.6 to 4.8 seconds; the latent period in the case of clam eggs (*Mya arenaria, Mercenaria mercenaria*) is from 8 to 9 seconds at

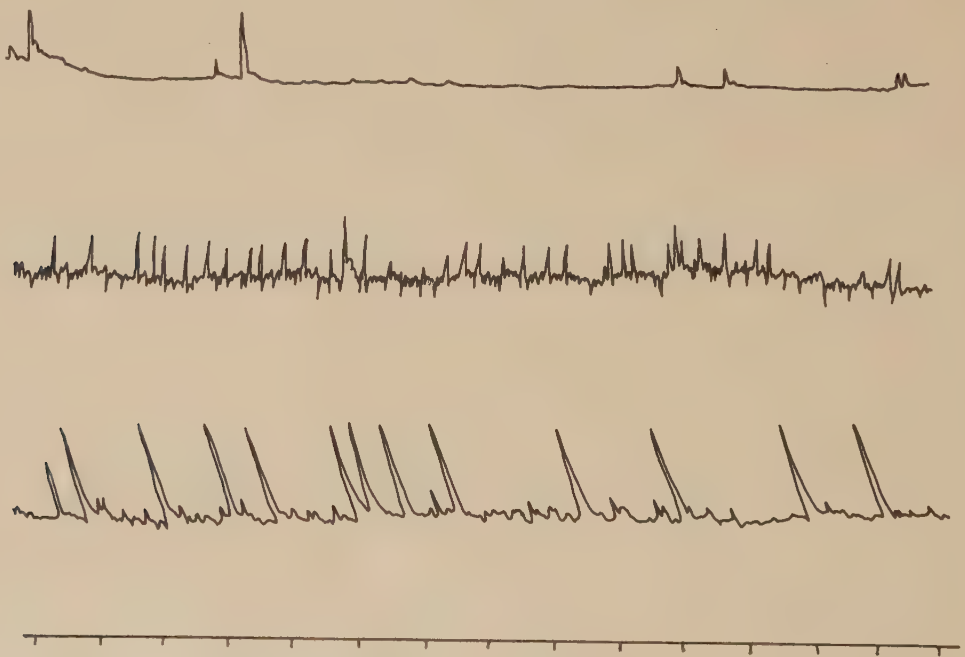


FIGURE 282.—Shell movements of three males of *C. virginica* recorded during the shedding of sperm. There was no change in muscular contraction before, during, or after spawning. Temperature 23.5° C. Time interval, 1 minute.

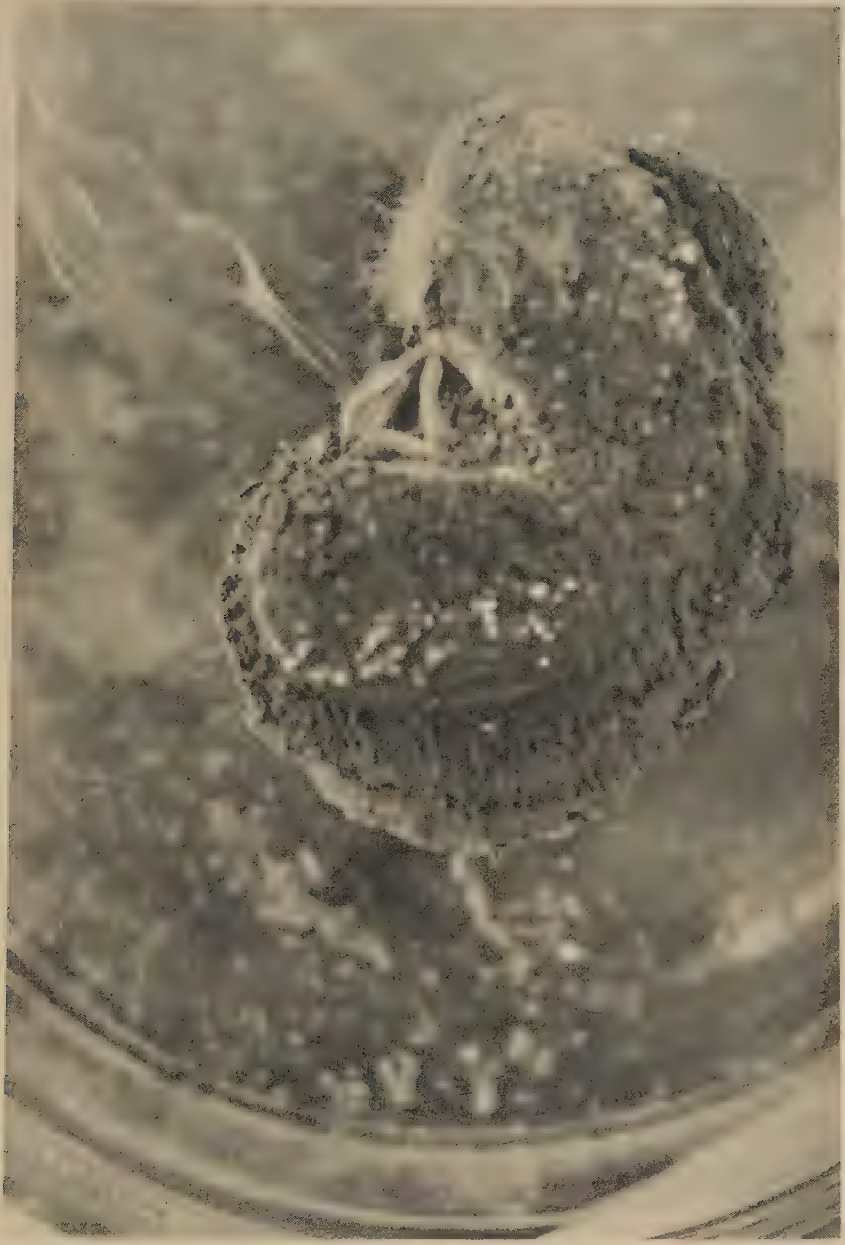


FIGURE 283.—Photograph of the spawning male, *C. virginica*. The V cut in the left valve was made several weeks before the experiment. Temperature 23.0° C. Sperm ejected through the opening of the promyal chamber is seen as a white jet opposite the V cut.

24° to 25° C. Many other substances including various hormones (thyroxin, adrenalin, estrogen), desiccated anterior and posterior pituitary, thymus, thyroidin, cysteine, glutathione, peptone, egg albumen, urea, different sugars (dextrose, maltose, d-arabinose), starch, and yeast stimulate ejaculation in various degrees of effectiveness. Suspension of desiccated thyroid gland (thyroidin) in sea water was found to be the most effective stimulant, and it has been used in the Woods Hole laboratory in preference to egg suspension or egg water.

Other substances may also provoke sexual response. Miyazaki (1938) found that the extracts from several algae, *Ulva pertusa*, *Enteromorpha* sp. and *Monostroma* sp. induce spawning in the males of *C. gigas*.

Mature males (*C. virginica*) respond also to live sperm of the species. In this case the latent period of spawning reaction is much longer, varying from 6 to 27 minutes at 20° to 21° C. The interesting fact is that in the case of sperm stimulating male spawning the latent period is of the same order of magnitude as that of the female spawning reaction. Possibly the sperm acts as a stimulant only after it has been absorbed by the oyster, while eggs and egg water act upon the receptors located on the body surface.

The active principle of sperm of *C. virginica* can be extracted with ethyl alcohol and benzene. The residual powder of the extract can be mixed with sea water and added to the gills of the female to induce a typical spawning reaction (Galtsoff, 1940). Spermatozoa of *C. virginica* carry another hormonelike substance which may be recovered after alcohol extraction. The substance was named "diantlin" by Nelson and Allison (1940), who found that it dilates the ostia and stimulates the increase of water flow through the gills.

FREQUENCY OF SPAWNING

Under laboratory conditions male oysters may be induced to spawn many times at very brief intervals. It is, therefore, reasonable to assume that they behave in a similar way in their natural environment. The females spawn only a limited number of times within one breeding period. Out of several hundreds of oysters tested in the laboratory, the majority of the females were induced to spawn two or three times within a 6-week period (July–August), and only one spawned seven times. Similar conditions exist with *C. gigas*. Frequently a substantial percentage of females of these two species fail to shed eggs in spite of a ripeness of their ovaries and favorable environmental conditions. The spawn may be retained in the gonads until late fall when it is reabsorbed.

The number of times the adult female oysters spawn under natural conditions can only be surmised from examination of gonads and the occurrence of larvae in plankton. It is very difficult to decide from plankton observations whether the entire oyster population spawned several times or if different groups of oysters produced the larvae appearing at intervals in plankton. In Long Island Sound, for instance, four or more "waves" in the occurrence of straight hinge larvae were recorded (Loosanoff and Nomejko, 1951a) in 1943, but the described periodicity may have been due to spawning of different populations living in shallow and deep water. Inasmuch as the laboratory experiments show that repeated spawning may be induced in the same female, it is reasonable to infer that in their natural habitat oysters spawn more than once during every breeding season.

Laboratory observations show that spawning of sexually mature *C. virginica* is sometimes inhibited and that the oysters fail to respond to all known methods of stimulation. Similar conditions exist with *C. gigas*, which sometimes fail to spawn in spite of full gonad development. Artificial stimulation by suspension of sex cells may facilitate spawning but is not always successful. The reason for this inhibition of spawning in sexually ripe oysters has not been established, but the work of Lubet (1955) on *Chlamys* and *Mytilus* throws some light on the problem. Lubet discovered that the excision of cerebral ganglia in these mollusks provokes precocious spawning and that the mutilated animals spawn much earlier than the controls. Excision of the visceral ganglia seems to retard spawning. These experiments suggest that spawning is under the control of the nervous system. It also appears significant that neurosecretion in the ganglia cells precedes gametogenesis and that maximum accumulation of the neurosecretory products occurs at the time of the maturation of sex cells. In the species studied by Lubet the neurosecretory granules were absent in the ganglia of the recently spawned out animals. Whether Lubet's findings on neurosecretion apply to sexually mature oysters is not known, but his work seems to indicate that in the bivalves he studied, the release of sex cells was facilitated by the removal of internal inhibition (excision of cerebral ganglia) and that the disappearance of the neurosecretory products from the cerebral ganglia was necessary for the mollusk to become receptive to spawning stimuli. The latter inference is based on the observation that partial disappearance of neurosecretory granules always occurs a few days before spawning. After the completion of spawning all neurosecretory cells are empty. These findings are not in accord with the results of studies conducted by Antheunisse (1963) on zebra mussels (*Dreissena polymorpha* Pallas) from the Amstel River near Amsterdam. The mussels were collected once a month, from November 1957 to November 1958, for histological examination. For extirpation experiments only adult females were used during the spring and summer of 1959. Antheunisse states that in spite of the parallelism between the neurosecretory and reproductive cycles in zebra mussels spawning and neurosecretion are not interrelated. Extirpation experiments were difficult to perform, and most of the mussels died some days after the operation. It is therefore apparent that further studies are needed to determine the role of the neurosecretion in reproduction of mussels, oysters, and other bivalves.

FECUNDITY OF THE OYSTER

The intensity of spawning as judged by the number of eggs or spermatozoa discharged in each instance is variable. In both sexes the number of sex cells produced by a ripe female or male depends on the size of the oyster and the degree of development of the gonad. The range of variation is enormous. If the female gonad is poorly developed, only a few thousands of eggs may be released. On the other hand, the number of eggs produced and discharged by a well-developed gonad may reach many millions. Potential capacity of the ovary, i.e., the total number of eggs produced by a female during the breeding season, is not indicative of its actual reproductive ability which is expressed by the number of eggs actually spawned. The following procedure is used for estimating the number of eggs released by the female. The oyster is placed in a 20 l. tank and spawning is stimulated by warming the water and adding sperm suspension. After the completion of spawning five samples of 100 ml. each are taken while the water is agitated by an electric stirrer. Eggs in the sample are killed by adding two to three drops of 1 percent osmic acid, allowed to settle, and are counted in a Sedgwick-Rafter chamber. The oysters used in four separate tests varied from 9.2 to 13.3 cm. in height. The number of eggs (in millions) discharged in one spawning were 15, 30.3, 70.3, and 114.8 (Galtsoff, 1930b). After discharging over 100 million eggs the last oyster had a gonad about 5.5 mm. thick containing vast numbers of eggs.

The results of these counts were questioned on the basis that the computed volume of the discharged eggs exceeds the total volume of the body (Burkenroad, 1947). Rechecking the data confirmed my estimate. The counts are correct within ± 10 percent, the principal source of error being the difficulty in obtaining uniform distribution of eggs in the tank.

In the ovary the eggs are tightly packed and compressed; upon their release the diameter of their rounded part is increased. The spawned eggs in the above tests averaged 40 μ in diameter. The volume of a given number of eggs can be computed by using the conversion table from diameters to volumes of spheres given in Perry (1941). Since the volume of one egg of 40 μ diameter is 33,510.3 μ^3, the volume of 115 millions of eggs, solidly packed would correspond to 3.8 cm.^{3} With an allowance of 25 percent for interspaces the volume of spawned eggs in the ovary would be about 4.8 cm.^{3} The latter figure is within the range of magnitude of the volume of the gonad obtained by the displacement method.

Not all the ovocytes become mature at the same time. During the intervals between spawning some of them grow and replace those discharged by the preceding ovulation.

The fecundity of *C. gigas* is even greater. The five large oysters of this species forced to spawn in the laboratory averaged 55.8 million eggs per oyster; post mortem examination showed that after ovulation they retained the major part of the gonadial material. In comparison to *C. virginica* and *C. gigas* the fecundity of the larviparous European oyster is rather low. Estimates of the mean number of larvae per oyster were made by Dantan (1913), Cole (1941), Cerruti (1941), and Millar (1961). In British waters the mean number of larvae vary from 90,000 for a 1-year-old oyster to over a million for a 4-year-old oyster. French oysters relaid in West Loch Tarbert, Scotland, after 1 year produced as many larvae as the native oysters on English beds. The number of larvae is dependent, of course, on the size of the oyster, as can be seen from the table given by Millar.

Diameter in cm.	Mean number of larvae estimated from five samples	Diameter in cm.	Mean number of larvae estimated from five samples
6.2–7.3	733, 400	7.4–7.6	1, 155, 000
7.3–7.4	1, 543, 000	8.3–8.5	616, 000

The fully grown *O. lurida* bear broods of 250,000 to 300,000 larvae, the number depending generally upon the size of the maternal oyster (Hopkins, 1936, 1937).

SEX RATIO, HERMAPHRODITISM, AND SEX CHANGE

The oviparous species of oysters of the genus *Crassostrea* usually are not hermaphroditic; specimens in which functional eggs and sperm are found together are relatively rare. This condition exists in *C. virginica*, *C. gigas*, *C. angulata*, *C. rhizophorae*, and is probably common to all members of the genus. The frequency of hermaphroditism in *Crassostrea* oysters varies with age and environment. The earliest record was made by Kellogg (1892), who found one hermaphrodite among the many adult *C. virginica* he kept in breeding tanks. Burkenroad (1931) reported that about 1 percent of the oyster population on the coast of Louisiana were hermaphrodites. Needler (1932a, 1932b) found only four hermaphrodites (less than 0.4 percent) among the 1,044 oysters of various ages growing on beds in the waters off Prince Edward Island. The hermaphrodites were found only among the 2-, 3-, and 4-year-old oysters; none were encountered in oysters from 5 to 8 years old. In the course of my studies I found only two hermaphrodites among several thousand sexually ripe oysters from 5 to 7 years old.

Amemiya (1929) reported only one hermaphroditic specimen among 120 sexually mature *C. gigas* (0.8 percent). The percentage is apparently higher in the Bombay oyster, *O. cucullata*, for Awati and Rai (1931) reported 23 hermaphroditic specimens (2.9 percent) among the 794 oysters they examined

The larviparous oysters of the genus *Ostrea* (*O. edulis*, *O. lurida*, *O. equestris*, and others) are as a rule ambisexual, i.e., they undergo rhythmical changes in sexuality. The initial phase in these species is usually male, followed by alternating female and male phases.

Orton (1927) distinguishes several arbitrary categories of sexual changes in *O. edulis* from pure male or female to hermaphrodites which contain an equal abundance of ripe spermatozoa and ova. Different transitional phases of sex changes which take place during the life of the European oyster are discussed later in this chapter.

Oysters have no secondary sexual characters, and their sex can be recognized only during the reproductive periods by microscopic examination of gonads. Sperm suspension, which can be forced out by gentle pressure on the surface of the gonad, is viscous and white; the suspension of eggs is creamy and has a granular appearance. Sex determination made with the naked eye should be verified by microscopic examination of smears.

In many species of bivalves sex is unstable, and hermaphroditism and alternation of sex are common. With respect to sex change oysters fall into two groups: oysters in which sexual phases change regularly in a definite rhythm, as in *O. edulis*, *O. lurida;* and those belonging to the *Crassostrea* type, in which the sexes of the adults are separate, as in *C. virginica*, *C. gigas*, *C. angulata*, and *O. cucullata*. The gonads of the oysters of the first group contain functional ova and spermatozoa simultaneously. These oysters are hermaphrodites. In the second group hermaphroditic individuals are relatively rare.

The difference between the two groups is not as explicit as it appears since the primary gonad of *Crassostrea* is bisexual, i.e., it contains the germinal cells of both sexes.

As early as 1882 the outstanding Dutch naturalist, Hoek (1883), in his studies of *O. edulis* made the important observation that "at the time when an oyster is sexually mature, it always functionates as a male as well as a female; it is, therefore, physiologically dioecious." The significance of this important discovery was appreciated nearly half a century later after Orton (1927, 1933) showed experimentally that maleness developed in 97.3 percent of young or adult females which carried eggs, embryos, or larvae. He further established the fact that the earlier state of maleness was always found in the more recently spawned females. Great advances in the understanding of sex changes in *O. edulis* and other species were made by the works of Stafford (1913) on *O. lurida*, by experimental research conducted by Spärck (1925), and particularly by observations on the American species made by Coe (1932–41). It was clearly established by these investigations that the young oysters of the larviparous species (*O. edulis*, *O. lurida*) become sexually mature first as males then gradually change into functional females; later they become males again, and such alternation with some modification continues throughout life. Comparable phases of sex changes occur in the *Crassostrea* species although the rhythm of sex alternation is different. At the age of 12 to 16 weeks the primary gonad of *C. virginica* is bisexual (ambisexual, according to Coe's terminology) since both ovogonia and spermatogonia are found in the same follicles

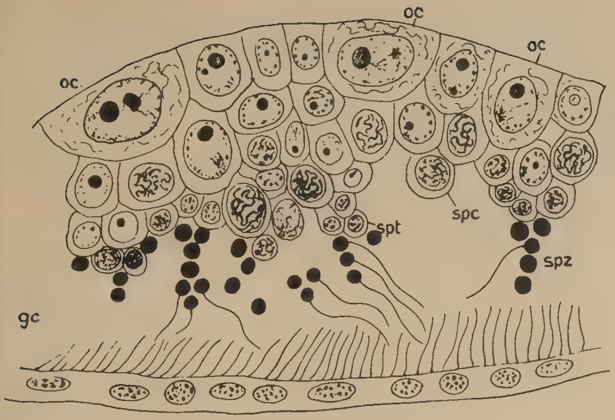


FIGURE 284.—Portion of bisexual gonad in young male *C. virginica.* gc—genital canal; oc—ovocytes; spt—spermatid; spc—primary spermatocyte; spz—spermatozoa. Photographically reproduced from Coe, 1934, fig. 5.

(fig. 284). At this stage the *C. virginica* gonad resembles that of *O. lurida* at the completion of the male phase and transition to female (fig. 285). In *C. virginica* the spermatogonia proliferate more rapidly than do the ovogonia and soon the young gonad attains a predominantly male appearance. Variation in the rhythm of gonad development in the oysters from different localities and even among those occupying the same bed results in different "categories" or "phases" of maleness or femaleness.

Development of the primary bisexual gonad in young *C. virginica* in New England waters is checked by the approach of winter when the growth of ovocytes is inhibited while the number of spermatogenic cells increases. A small number of spermatids may be formed early in November when the oysters are about 4 months old. The spermary of these secondary males contain scattered ovocytes, many of which degenerate, but some of them continue to develop into ova capable of fertilization. Even at the stage designated by Coe (1934) as "true male" the spermary at sexual maturity still retains a small number of ovocytes on the walls of the follicles.

At the close of the first breeding season many undifferentiated cells remain in the gonad to form the germinal cells of the following year.

Transformation of a bisexual gonad of *C. virginica* into an ovary begins before the formation of spermatozoa. At this stage the spermatogenesis is inhibited by the growth of ovocytes and the female phase is attained in a certain percentage of young oysters. The protandry, i.e., the development of maleness before the female phase, is well pronounced in *C. virginica.*

At their first breeding season young oysters form several sex classes: immature individuals in which the sex cells have not differentiated; males; hermaphrodites in which functional spermatozoa and ova are found in the gonad; and females. Hermaphroditic oysters are capable of self-fertilization and produce apparently normal larvae. The relative abundance of different sex phases of young oysters varies greatly, as can be seen from table 34,

TABLE 34.—*Frequency of different sex phases among* C. virginica *at first breeding season*

[According to Coe, 1934]

Locality	Immature	Hermaphrodite	Males	Females	Total examined
W. Sayville, N.Y	77	4	154	48	283
Great South Bay, N.Y	21	0	197	7	225
New Haven, Conn. (1932)	17	4	389	13	423
New Haven, Conn. (1933)	3	0	129	7	139
Woods Hole, Mass	373	3	9	4	389

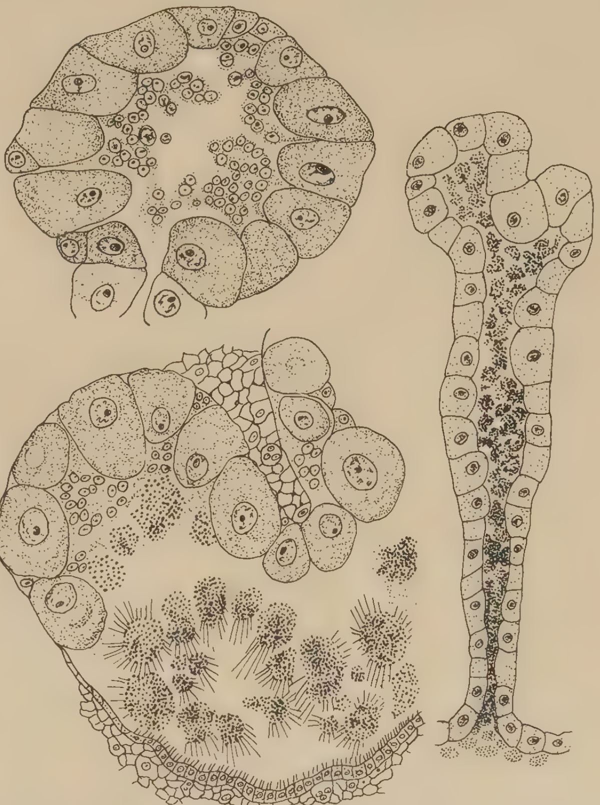


FIGURE 285.—Transition from male to female phase in *O. lurida.* Lower left—genital canal filled with sperm balls ready to be discharged; spermatogonia on the surface of large ovocytes. Right—advanced male phase in an older oyster; ovarian follicle is packed with cells in the later stages of spermatogenesis. Upper left—female phase preceding ovulation; many spermatogonia in the lumen of the follicle. Redrawn from Coe, 1932c.

which summarizes Coe's observations (1934) made on oysters from four different places along the coast of Massachusetts, Connecticut, and New York.

During the second breeding season the number of males may still exceed that of the females, but generally the sex ratio approaches equality. Great differences in the degree of protandry among the 1-year-old oysters is associated with the differences in the growth rate. Coe's observations suggest that there is a correlation between the development of ovocytes in the bisexual gonad and the rate of body growth. At the first breeding season the average size of the females is much larger than that of males (Needler, 1932a, 1932b). Coe (1934) found that the mean height of 389 yearling males from the New Haven area was 31.28 mm. (Std. dev. ±6.33) and that of the 13 females of the same age 38.54 mm. (Std. dev. ±8.12). The difference does not seem to be statistically significant, and several interpretations were advanced by Coe. He suggested that the females require more favorable conditions in order to mature, that they are metabolically more active, and that at the critical period of sex differentiation the metabolic factor determines the predominance of the male or female cells in the primary bisexual gonad. These proposals require corroboration. Since it is known that the growth rate of young oysters is accelerated by keeping them suspended above the bottom, there apparently would be no difficulties in conducting a comparative test using slow and fast growing oysters selected from a single population.

After spawning the gonad of *C. virginica* retains its bisexual potencies and its sex may alternate in either direction. Needler (1932a, 1932b) was the first to demonstrate that such a change actually occurs among adult *C. virginica*. She found that out of 24 surviving oysters which were known to be males during one summer, 5 became functional females the following year, and out of 12 females 5 changed to males. Among the 57 *C. virginica* studied by Needler (1942) for a period of 4 years there was a high proportion of males which remained unchanged while other oysters changed sex at least once. Some of the individuals changed sex every year. Needler suggested, without providing corroborative evidence, that the sex of the males which remained unchanged was genetically determined and that the other oysters in which sex alternation occurred at random were hermaphrodites.

Adult Japanese oysters, *C. gigas*, may change their sex during the interval between the two breeding seasons. Amemiya (1929), who established this fact, found that the rate of change was higher in the males (60 percent) than in the females (25 percent). Sex change occurs also in *C. commercialis;* 95 percent of the very young oysters of this species were found by Roughley (1933) to be males, but among the adult specimens of large size the females predominated at the rate of 270 to 100 males.

The sex of the oysters used in the investigations by Needler and Amemiya was determined by drilling a hole in the shell and pinching off a piece of gonad for microscopy. The injury caused by the operation constitutes a factor which may affect the unstable gonad and influence its sex change. By removing about one-third of all gill lamellae in adult *C. gigas* at the time when the gonad was at the indifferent phase after spawning, Amemiya (1936) demonstrated that the percentage of males in the mutilated group in all cases was larger than those in the control. Removal of the gill tissue may have indirectly influenced the development of male sex by reducing the rate of feeding and growth. This assumption also needs further corroboration.

Injury to the oyster used for observation on sex change can be avoided by inducing spawning in each individual oyster, obtaining kymograph tracings of muscular contractions, and examining the discharged sex cells. This technique was employed in the Woods Hole laboratory. In a test which continued for 5 consecutive years, 4-year-old oysters were obtained from one of the private oyster beds near Onset, Mass. During the first summer 202 oysters were induced to spawn, their sex was recorded, and an identifying number was engraved on the right (upper) valve. Upon completion of the tests the oysters were returned to outdoor tanks or were placed in the harbor and remained there until the next reproductive season. The testing was repeated every summer (Galtsoff, 1961).

Because the males respond to spawning stimuli more readily than the females, their number at the beginning of the experiment was greater than that of the females. The disparity does not represent an actual sex ratio of the population of 4-year-old oysters which was found to be about 1 to 1. The mortality, especially among the 7-year-old oysters used in the test, was high, and the number of nonspawning oysters gradually increased toward the end of the experiment (table 35).

When oysters failed to respond to spawning stimulation, the testing was repeated at 4- to 5-day intervals for 5 consecutive weeks. Negative results were assumed to indicate the oysters were nonfunctional sexually, and they were returned to the holding tanks for another year. In several instances the oysters that failed to spawn became sexually active the following breeding season. It is not known at present whether the increased number of failures to spawn and increased mortality (table 35) should be attributed to aging or to unfavorable conditions in the winters. On several occasions the holding tanks and live cars in which the oysters were kept were swept by stormy waters and everything inside was covered with a deposit of mud.

TABLE 35.—*Changes in the percentage of sexes in a selected group of* C. virginica *tested consecutively for 4 years*

[Initial number of males and females 4 years old does not represent the normal sex ratio which was about 1 to 1]

Age in years	Males	Females	Non-spawning	Total survivors
Number	*Percent*	*Percent*	*Percent*	*Number*
4	64.4	35.6	0	202
5	65.2	32.6	2.2	181
6	61.2	31.6	7.2	139
7	50.1	40.3	9.6	104
8	44.1	41.2	14.7	68

During the 5-year period of observations the number of spawning males decreased from 119 to 15 in 9-year-old mollusks. The number of females decreased from 63 to 18. Consequently the sex ratios of males to females of the experimental oysters changed from 1.9:1 at the beginning to 0.8:1 at the end of the observations. The predominance of oysters of female sex in the surviving oysters can not be attributed to more frequent sex changes from male to female. In table 36 no significant differences were recorded in the rates of sex alternation in the two groups. The predominance of females at the end of the test could be explained, therefore, by greater survival rate of oysters at the female phase. This interesting point requires further corroboration.

Out of the 68 survivors at the end of the breeding season of the fourth year, 31 had alternated their sex at least once during the period of testing (Galtsoff, 1961). The frequency of changes were as follows:

One change	18 instances.
Two changes	10 instances.
Three changes	2 instances.
Four changes	1 instance.

The oyster which changed sex every year was a male at the beginning and returned to the male phase at the last test. No distinct pattern is apparent in the rhythm of changes except the greater persistence of the female phase. The sex ratio within the sex-reversed oysters changed from 23 males and 8 females at the beginning to 11 males and 20 females at the end of the observations.

TABLE 36.—*Frequency of sex alternation in adult* C. virginica *from 5 to 9 years old*

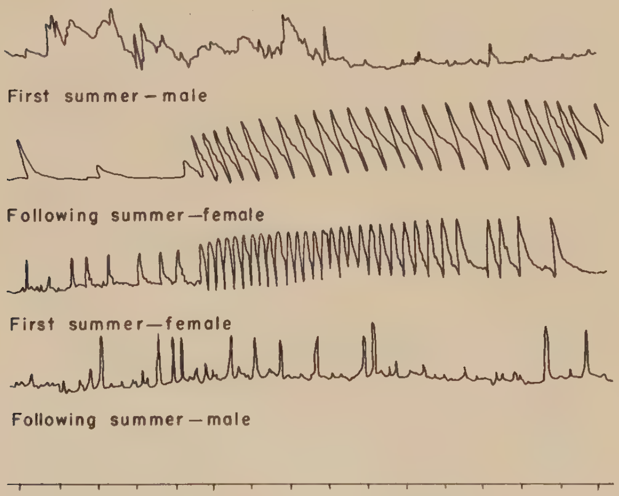
[The figures in the table indicate the number of males (column 2) and females (column 5) of each age and the number and percentage of changes to females (columns 3 and 4) and to males (columns 6 and 7) that occurred during each year]

Age in years	Males	Changes to females		Females	Changes to males	
Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
5	119	10	9.2	63	11	17.4
6	88	10	11.3	38	9	23.7
7	65	15	23.1	33	3	9.1
8	25	3	12.0	24	6	25.0
9	15	1	6.7	18	1	5.6

The cause of sex instability and the factors which may influence the shift of a gonad from one sex to another are not known. Coe believed that the physiological state of the organism in each breeding season is the key to the determination of the sexual phase of the oyster. No concrete proof to substantiate this idea can be found in his experiments. Egami (1952) attempted to transplant pieces of gonad of *C. gigas* to another oyster of the same or of the opposite sex. He found no evidence that the sex of the host affected the sex differentiation of the graft and concluded that the sex of the grafted pieces has been determined at the operating season (December) at their morphologically undifferentiated state. In another work (Egami, 1953), he corroborated the results of Amemiya's observation on the decrease of growth rate of *C. gigas* by the removal of gill tissue and the increase in the percentage of males among the mutilated oysters. He concluded that maleness could not be attributed directly to mutilation but was associated with the decreased growth rate of oysters without gills. Egami found that among normal individuals of *C. gigas* those growing more rapidly during the autumn tended to develop into females the following reproductive season.

The conclusions may be considered only tentative since they are based on a small number of experiments which need to be repeated on a larger and more comprehensive scale. Sex alternation in oysters offers fascinating possibilities for further research on this fundamental biological problem.

The spawning reactions of sex-reversed oysters, as reflected in the type of shell movements and in the manner of dispersal of sex cells, are in every respect identical to those of the reactions of true males and females (fig. 286). In some sex-reversed oysters the change from male to female behavior is however delayed. Examination of records shows that in several instances females which were males during the preceding year spawned at the beginning of the reproductive season in a male fashion, discharging the eggs through the cloaca. Full female reaction involving rhythmical contractions of the adductor muscle was fully developed toward the end of the spawning season (fig. 287). It can be inferred from these observations that the mechanism of female sexual behavior develops at a slower rate than the morphological changes in the gonads. There was no retention of female behavior in oysters which returned to the male phase. The male reaction was apparent in them at the beginning of the season.



Figures 286.—Kymograph records of sex-reversed male (two upper lines) and sex-reversed female (two lower lines) *C. virginica* recorded at two consecutive breeding seasons. Both oysters were 5 years old at the "first" summer. In both instances eggs were dispersed through the gills, the sperm through the cloaca. Time interval, 1 minute.

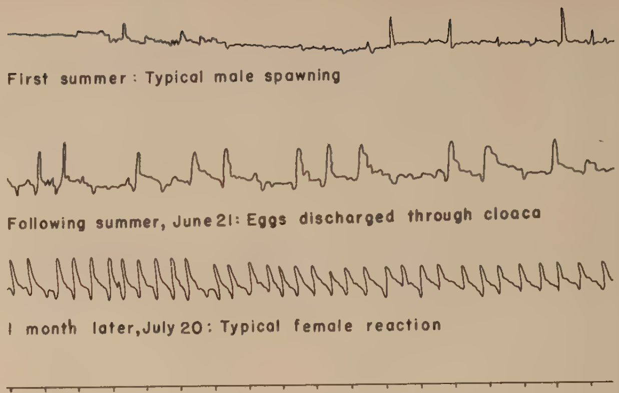


Figure 287.—Spawning of the sex-reversed male *C. virginica.* First line—spawning at the male phase. Second line—sex of the gonad changed to female; spawning proceeds in the male fashion. Third line—typical female spawning. Oyster was 5 years old during the "first" summer. Time interval, 1 minute.

In one instance the spawning of an hermaphroditic oyster was recorded (Galtsoff, 1961). Both eggs and sperm were discharged simultaneously through the cloaca, and the rhythmical contractions of the adductor muscle were not fully developed (fig. 288). A small portion of the gonad of this oyster is shown in fig. 289. Microscopic examination of the tissue revealed the presence of relatively few mature eggs in the follicles occupied by spermatozoa. Spawning of this oyster was induced by raising the water temperature. Eggs removed from the spawning tank were found to be fertilized and their development traced to trochophore stage was normal.

LUNAR PERIODICITY

The modern zoologist may disregard the early popular beliefs and superstitions which endowed the moon with mysterious effects on human affairs, on animals, and plants; nevertheless, he is confronted with several undeniable instances of lunar periodicity in the reproduction of marine invertebrates. Probably the most famous and generally known examples are the swarming and breeding of the Palolo worm (*Eunice viridis* Gray) of the South Pacific at the moon's last quarter of

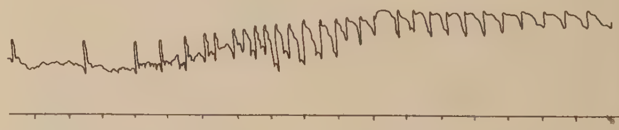


Figure 288.—Shell movements of a spawning hermaphroditic *C. virginica.* Temperature 24.5° C. Time interval, 1 minute.

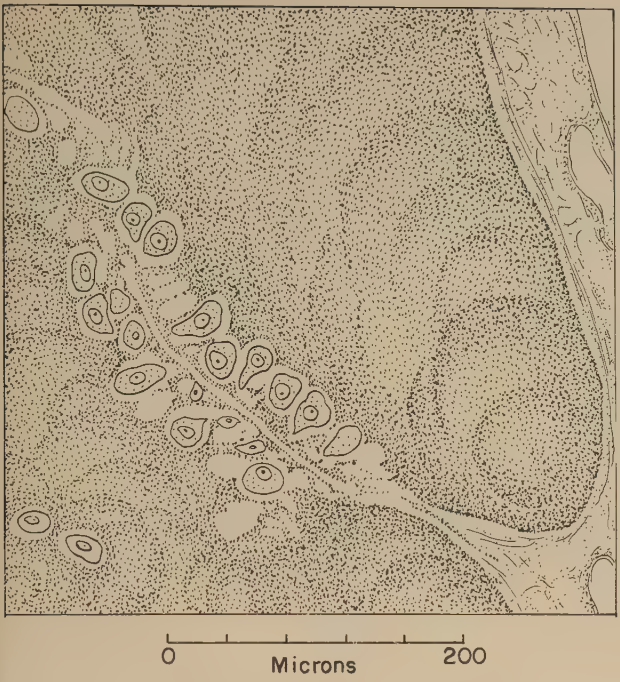


FIGURE 289.—Section of a small portion of an hermaphroditic gonad of *C. virginica.* Bouin, hematoxylin-eosin.

October and November; the swarming of the Atlantic Palolo (*Odontosyllis enopla* Verrill) at Bermuda and *Eunice fucata* Ehlers, at Tortugas, Florida (Mayer, 1908); and the breeding habits of *Heteronereis* form of *Nereis limbata* at Woods Hole (Lillie and Just, 1913). Legendre (1925) gives an interesting historical account of the effect of the moon on marine organisms. A comprehensive review of the instances of lunar periodicity of breeding among many marine invertebrates, including several species of pelecypods, is given by Korringa (1947).

Evidence of a relationship of breeding of *O. edulis* to moon phases was first presented by Orton (1926), who examined weekly samples of adult oysters from Fal estuary and found two important maximums in spawning at the full moon spring tides in the year 1925. He further concluded that the population as a whole gave maximal percentage of spawn (based on presence of embryos in the oysters) in the weeks after the July and September full moons. Later observations by Korringa (1941, 1947) in the commercial oyster district of Oosterschelde, Holland, confirmed the existence of a relationship between breeding of *O. edulis* and moon phases. He found that the full moon exercises the same influence on the breeding of oysters as does the new moon. Korringa based his studies on determinations of the time and abundance of oyster larvae in plankton and found a marked periodicity in the maximums of oyster larvae occurring about 10 days after full and new moon. Fluctuations in water temperatures, according to Korringa's view, are apparently of little or no importance in causing the periodicity in swarming which appears to be correlated with the spring tides. Unfortunately no experimental evidence is available to substantiate this inference which is based entirely on the concurrence of the two phenomena.

Spawning of *C. virginica* has no relationship to lunar phases. The existence of such a relationship was postulated by Prytherch (1929), who stated that Long Island Sound oysters spawn "at the end of full moon tidal period, or eight days after the time of full moon," but the correlation could not be corroborated by careful studies of Loosanoff and Nomejko (1951a), who continued the observations in Long Island Sound over a period of 13 years after the termination of Prytherch's work. Negative results were also reported by Hopkins (1931) in Galveston Bay, Tex., and by R. O. Smith in South Carolina waters (unpublished reports on file in the Bureau of Commercial Fisheries).

BIOLOGICAL SIGNIFICANCE OF SPAWNING REACTION

The most outstanding single factor in oyster reproduction is the difference in spawning behavior of the two sexes. The males are more responsive to sexual stimulation than the females and are easily stimulated to spawn by rising temperatures and by a great variety of organic substances, some of them not found in natural sea water. The spawning response of the male is nonspecific.

The less responsive, sexually mature females require stronger stimuli and are highly specific to chemical stimulation; they respond only to suspensions of sperm of the same or related species and are indifferent to the sperm of other bivalves and various chemical substances tested. The specificity of females is an insurance that eggs cannot be discharged when there is no sperm in the water.

Males are usually the first to initiate spawning; the discharge of sperm by even one individual induces spawning by those next to it, and the process spreads over the entire oyster community. This sequence has been observed among oysters

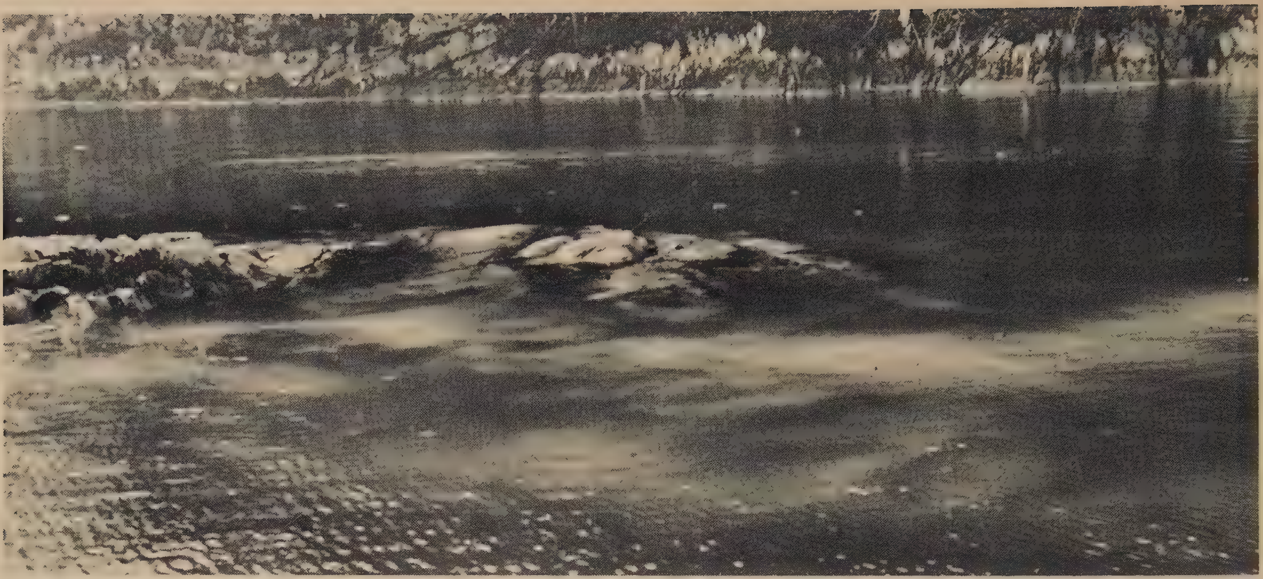


FIGURE 290.—Spawn of *C. gigas* carried down by the tidal river near Vancouver, British Columbia. Courtesy of D. Quayle. Black and white enlargement of Kodachrome slide.

kept on floats and among specimens living under natural conditions on oysters beds near low tide level. The spawning of an entire oyster population can be artificially initiated by mincing the meats of several sexually mature oysters and spreading them into the waters of the oyster bed. This method, based on my laboratory experiments, has been applied on a commercial scale by oyster growers in British Columbia (Elsey, 1936).

Simultaneous spawning of oyster populations is essential for the production of a large brood of oyster larvae and for obtaining setting of commercial value. In the estuaries where the majority of oyster beds are located, the tides carry the released spawn for some distance before the eggs sink to the bottom. A transport of oyster spawn by a strong current can be seen in the photograph (fig. 290) taken by Quayle near Vancouver, British Columbia, and kindly given to me for reproduction. The white streak in the foreground of the clear river water was formed by billions of eggs and sperm discharged by a population of *C. gigas* several miles up river.

The method of discharging sperm and eggs is also of considerable significance. Spermatozoa carried away by the respiratory current remain in suspension for several hours. When eggs are discharged in the same manner by some sex-reversed oysters, they rapidly sink to the bottom only a few inches away from the female. Laboratory observations indicate that under such conditions only a very small percent of them are fertilized or have even a slim chance of developing. On the other hand, eggs discharged in the usual manner through the gills and forcibly ejected from the mantle cavity, have a much better chance to be fertilized and survive. Furthermore, because of the specificity of female response to sperm, eggs are ejected only when the water contains free spermatozoa of the same species. The female spawning reaction is an adaptation of an oviparous organism to the conditions of its existence and assures the survival of the species.

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CHAPTER XV

EGG, SPERM, FERTILIZATION, AND CLEAVAGE

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As early as 6 to 10 weeks after setting, young *C. virginica* of New England waters, then 6 to 8 mm. in height, develop primordial gonads of profusely branching tubules (Coe, 1932a). At this stage the germinal epithelium is a layer of morphologically undifferentiated cells; some of them will transform into larger cells to become ovocytes, i.e., the cells destined to develop into mature eggs. The smaller cells of the epithelium proliferate very rapidly and are recognizable as the male germ line, and eventually develop into spermatozoa. For several weeks the immature, or primary, gonad of an oyster remains nonfunctional and bisexual (ambisexual), for it contains both male and female germ cells which will transform into mature spermatozoa or ova during the following summer. In some individuals the primary bisexual gonad is retained until the second year, a delay which Coe (1932a, 1938) attributes to poor nutrition.

The more rapid multiplication of male germ cells suppresses the development of ovocytes and results in a predominance of males among the 1-year-old oysters and in the appearance of different degrees of intersexuality (predominance of the cells of one sex over the other). In the same brood which contains also distinctly ambisexual oysters there are, however, other young individuals in which the primary gonad develops directly into ovary or spermary. Local conditions on oyster beds apparently influence the tempo of changes. In the warmer waters at Beaufort, N.C., young oysters are more apt to develop directly into females than in the northern cold waters of New England. Coe (1938) found that the proportion of females to 100 males varied at the first breeding season between 37.1 and 48.8 at Beaufort; 5.6 and 24 at Milford, Conn.; and 3.3 and 12.5 at New Haven Harbor. The differences are not consistent with geographical latitude since the female to male ratio at West Sayville, Long Island, N.Y., was 31.2; at Delaware Bay 41.9; and at Apalachicola, Fla., 7.1. It is obvious that these variations cannot be attributed to temperature alone and are probably caused by a combined effect of environmental conditions.

Toward the end of the second breeding season the primary gonad is transformed into a definite ovary or spermary (fig. 291). The gametogenesis, i.e., complete transformation of the primordial germ cells into mature ova (ovogenesis) or spermatozoa (spermatogenesis), is a very complex process. The differentiation is accompanied by rapid multiplication of the new generations of cells which

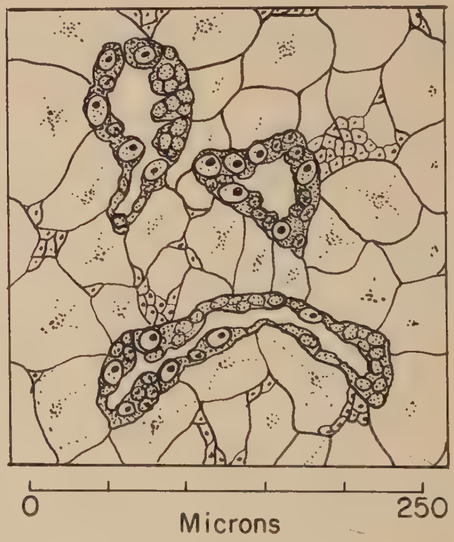

FIGURE 291.—Section of a gonad of *C. virginica* at an early stage of differentiation. End of March, Woods Hole, Mass. The larger, clear cells are ovogonia, the smaller ones are undifferentiated cells of germinal epithelium. Bouin, hematoxylin-eosin.

extend inward and fill up the lumen of the follicles. Early at this stage the sex cells become dense and opaque, a condition which interferes with cytological study.

Gametogenesis of *C. virginica* and *O. lurida* has been studied by Coe (1932a, 1932b, 1934, 1936, 1938) and that of the Australian rock oyster, *C. commercialis*, by Cleland (1947). Only the main points of this process were disclosed by these investigations.

OVOGENESIS

In all animals the primordial germ cells become distinguishable as primary ovogonia in the females or spermatogonia in the males after a certain number of divisions. After a period of quiescence they begin to divide again and give rise to secondary ovogonia or spermatogonia. After several generations the cells stop dividing and enter a growth period, which is more prolonged in the females than in the males. The growth period is characterized by a series of cytological changes, each differing from the preceding stage. The cells which will produce gametes are at this stage called auxocytes, from the Greek "auxesis" meaning growth, and are referred to as ovocytes in the female and spermatocytes in the male.

Ovogenesis in oysters begins with the appearance of enlarged cells in the germinal epithelium. These are the ovogonia, which in *C. virginica* and *O. lurida* are distinguished from other cells of the germinal epithelium by their relatively large nuclei with conspicuous nucleoli and loose chromatin network (fig. 292, og). The ovogonia usually lie next to the follicle wall, and their distal sides do not protrude into the lumen. Differences between the early ovogonia and indifferent residual cells (I) are not conspicuous. Examination of a series of sections and study of the sequence of changes in the appearance and structure of the cells are necessary to assure a positive identification.

After one or two divisions the ovogonia change in appearance as well as in size. This generation of female sex cells called ovocytes can be recognized by the presence of fibrillar mitochondrial bodies (sometimes called yolk nuclei), and by the spiremes of densely packed chromosomes (figs. 293 and 294). Their nucleoli become very conspicuous.

During the last stage of ovogenesis the ovocyte begins to grow rapidly, and the distal part, grossly enlarged and rounded, protrudes into the lumen of a follicle. At the same time the connection

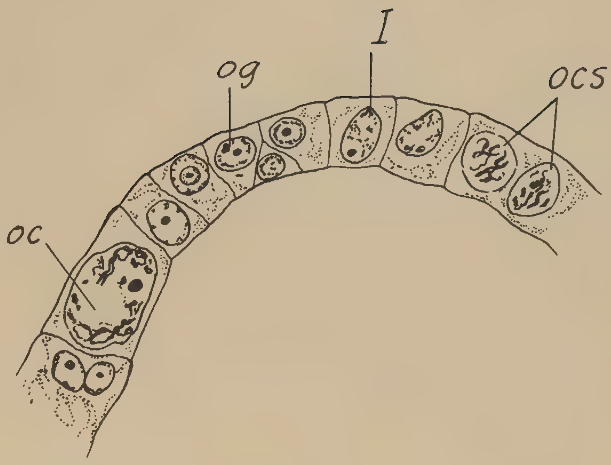

FIGURE 292.—Follicle wall of the ovary of *C. virginica*. I—indifferent residual cell; oc—residual ovocyte; ocs—two young ovocytes in synaptic phase; og—group of ovogonia. Redrawn from Coe, 1932a, fig. 9. Highly magnified.

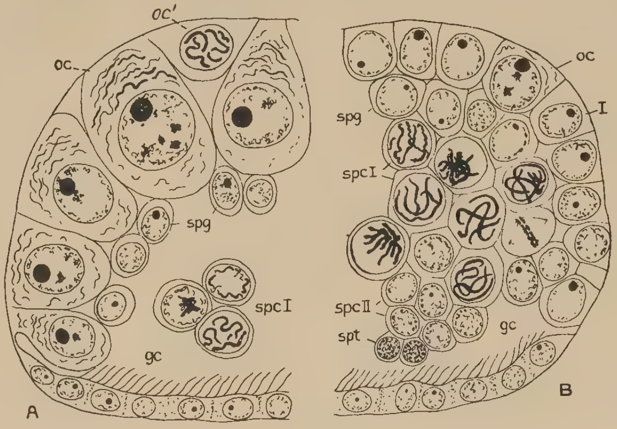

FIGURE 293.—Portions of two follicles of a bisexual gonad of 4-month-old *C. virginica*. A—predominantly female, and B—predominantly male follicle; gc—genital canal lined with ciliated cells; oc—large ovocyte; oc^1—young ovocyte in spireme phase; spcI—primary spermatocytes in spireme phase; spcII—secondary spermatocytes; spt—spermatides. Photographically reproduced from Coe, 1932a, fig. 6. Highly magnified.

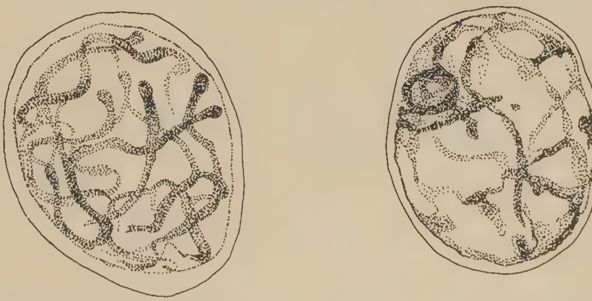

FIGURE 294.—Two young ovocytes at spireme stage in a mature ovary of *C. virginica*. Redrawn from Coe, 1932a, fig. 9. Highly magnified.

with the basal membrane of the follicle wall is narrowed to an elongated stem. The nucleus increases greatly in bulk, and the developing egg assumes a pear-shaped form. An accumulation of dark granules (mitochondria) at the proximal end of the cells may indicate that food for the growing ovocyte is obtained through the wall of the follicle. The granules are not pronounced in the ovocyte of *C. virginica* but are conspicuous in some other bivalves, particularly in *Sphaerium* (Woods, 1932). From the beginning of sexual differentiation to the final maturity of an ovum, the early ovocyte increases in volume more than 3,000 times.

Ovogenesis in the Sydney rock oyster *C. commercialis*, described by Cleland (1947), is somewhat different from the ovogenesis of the American species. At the earliest stage before the start of the growth phase an ovocyte of the rock oyster is a small cell, 4 μ to 5 μ in diameter. Two-thirds of the cell is occupied by a clump of chromosomes surrounded by a rim of clear cytoplasm. Cleland identifies this stage as a definite auxocyte, i.e., an ovocyte just before entry into the growth phase. At ths stage the cell has no nucleolus.

The definite auxocyte begins to grow and passes through three stages (called by Cleland Auxocyte I, II, and III) which differ in size and nuclear structure. Auxocyte I has a diameter of about 9 μ, with a relatively large germinal vesicle (6 μ) and a nucleolus of about 1.7 μ. The nucleus is centrally placed in the homogeneous cytoplasm with an excentric nucleolus which is not in contact with the nuclear membrane. Auxocyte II has diameter of 12.6 μ, with the germinal vesicle (nucleus) about 7 μ and nucleolus 2 μ to 3 μ. The cell is usually spherical with a centrally located germinal vesicle and chromosomes spaced more widely than in Auxocyte I. At this stage a group of granules appears at one pole of the nucleus. Auxocyte III is a spherical cell 20 μ in diameter, with the germinal vesicle measuring 11 μ and eccentrically located nucleolus of about 4.2 μ in diameter. The cell is separated from the wall and is free in the lumen of a follicle. Protein granules are abundant along the periphery of the cell where they are found in a mature egg. The mature ovocyte of *C. commercialis* has a diameter of about 38 μ. The nucleus is large, about 21 μ across; the nucleolus is 4.6 μ. The chromosomes are paired and are usually placed peripherally in the germinal vesicle but not in contact with the nuclear membrane. Crossing-over is frequently seen at this stage but the chromosomes are not coiled.

SPERMATOGENESIS

Spermatogenesis in the oyster is known primarily from the studies by Coe (1931) on the development of the gonad of young *O. lurida*. Comparison with the gonads of *C. virginica* shows that there is close agreement in the general features of the process in both species. Progressive stages of the formation of sperm begin with the undifferentiated gonia which line the inner wall of the gonad follicles. After a large number of descendants have been produced the spermatogonia can be distinguished from the ovogonia by their smaller size and position within the follicles. Ovogonia lie in a single row along the wall; the primary spermatogonia of *C. virginica* are found either singly or in groups between the ovogonia lining the wall and in the lumen (fig. 295).

In the hermaphroditic gonad of *O. lurida* a single primary spermatogonium divides several times to form a cluster of cells which become separated from the follicle wall and occupy a position toward the center of the lumen (fig. 296).

The number of divisions of spermatogonia presumably depends on the amount of nourishment available to the gonad. Coe estimates that in *O. lurida* each primary spermatogonium divides six to nine times to produce a cluster of 64 to 500 cells. In spite of the close contact the adjacent cells of the clusters are separate but are held together by a delicate noncellular secretion. Its

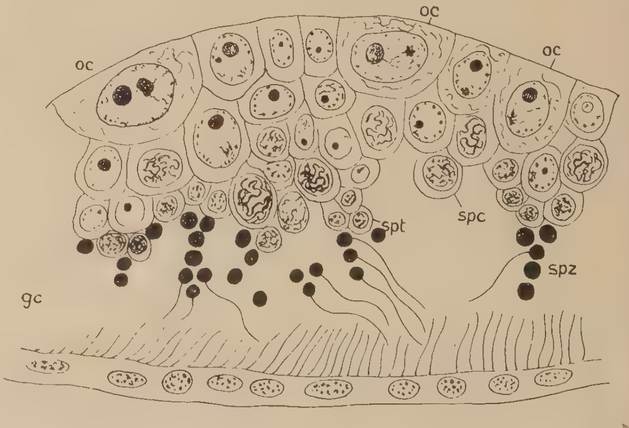

FIGURE 295.—Portion of bisexual gonad of young *C. virginica*. gc—genital canal; oc—ovocytes with spermatogonia filling the lumen; spc—spermatocytes; spt—spermatids; spz—spermatozoa. Photographically reproduced from Coe, 1934, fig. 5A. Highly magnified.

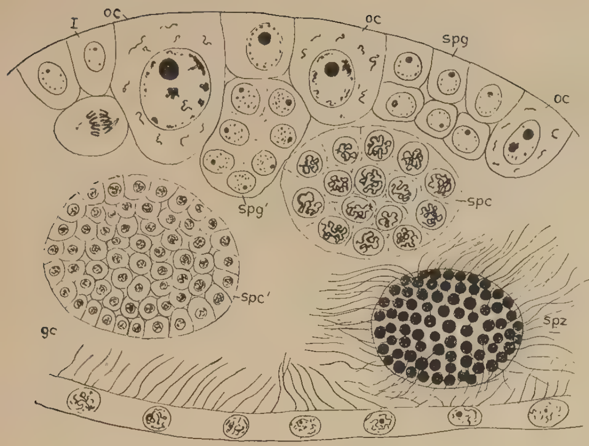

FIGURE 296.—Portion of an hermaphroditic gonad of *O. lurida*. gc—genital canal; I—indifferent cells; oc—ovocytes; spc—primary spermatocytes; spc^{1}—secondary spermatocytes; spz—spermatozoa united into a sperm ball. Photographically reproduced from Coe, 1934, fig. 5B. Highly magnified.

chemical nature has not yet been determined. In poorly preserved preparations the clusters sometimes have the appearance of syncytia with nuclei embedded in a common matrix. In both species all spermatogonia have a conspicuous nucleolus and loose chromatin reticulum. As the divisions proceed the diameter of the spermatogonia diminishes from about 6μ to 3μ or less at the last stage leading to the formation of primary spermatocytes. In *C. virginica* these cells are globular, each with a large nucleus resolved into slender threads (spiremes). This leptonema stage is frequently observed in the developing spermary but the conjugation of chromosomes (synapsis) has not been described with any detail. However, in reference to the spermatogenesis in *O. lurida*, Coe (1931) states that the leptotene stage "is followed by the usual process of synapsis."

The appearance of secondary spermatocytes is similar to that of the primary. In *C. virginica* they can be distinguished by their radial orientation in the lumen and small size. Different phases of spermatogenesis in *C. virginica* are shown in a semidiagrammatic drawing published by Coe (1932a) and reproduced in fig. 297. Meiotic divisions and the transformation of spermatids into mature spermatozoa have not been fully described for *C. virginica*. In a mature spermary the spermatozoa are always oriented with their tails toward the center of the lumen. The photomicrograph shown in fig. 298 shows the gradual increase in the number of male sex cells from the wall of the follicle toward the center. Successive stages of the spermatogenesis of *O. lurida* drawn by Coe are shown in fig. 299.

Secondary spermatocytes of *O. lurida* are held together in spherical masses. Close contact by the spermatids continues during their transformation into spermatozoa; in the sperm ball of a mature oyster the tails radiate from the center. Each sperm ball is composed of from 200 to 2,000 spermatozoa originating from a single spermatogonium (Coe, 1932b). During mitotic divisions the "prophase, metaphase, and telophase are all of typical appearance, with a delicate spindle of the usual form" (Coe, 1931). Because of the crowded condition of the metaphase and anaphase plates, Coe was unable to determine the chromosome number which he states "is not very large." In two diagrammatic drawings of spermatocyte division Coe (1931, fig. 3, E and F) figures 10 chromosome pairs. This is the most common number of chromosomes found by Cleland at the two- and four-cell stage of cleavage in the fertilized egg of *C. commercialis* (Cleland, 1947). The number of chromosomes seen during the cleavage of *C. virginica* eggs is discussed later (p. 345).

STRUCTURE OF THE MATURE EGG

Eggs in the mature ovary of *C. virginica* are pear-shaped and compressed. Many of them are attached to the follicle wall by long, slender peduncles; others are free in the lumen ready to be moved to the genital canals and discharged (fig. 300). The long axis of the eggs varies from 55μ to 75μ depending on their shape; the width at the broadest part measures from 35μ to 55μ, and the diameter of the nucleus is from 25μ to 40μ. The oblong shape is retained for some time after the discharge of eggs into water but gradually the egg becomes globular and denser. Under the transmitted light of a microscope the nucleus appears as a large, transparent area surrounded by densely packed granules (fig. 301). In a globular egg the nucleus cannot be seen unless it is cleared in glycerol or other clarifying reagents (fig. 302).

Eggs of oysters living under marginal conditions in water of salinity less than 10 ‰ frequently become cytolyzed upon their removal from the ovary; the nuclei appear larger than those of normal eggs. Only 1 or 2 percent of these eggs is fertilizable. The delicate primary or "vitelline" membrane surrounding the unfertilized egg is

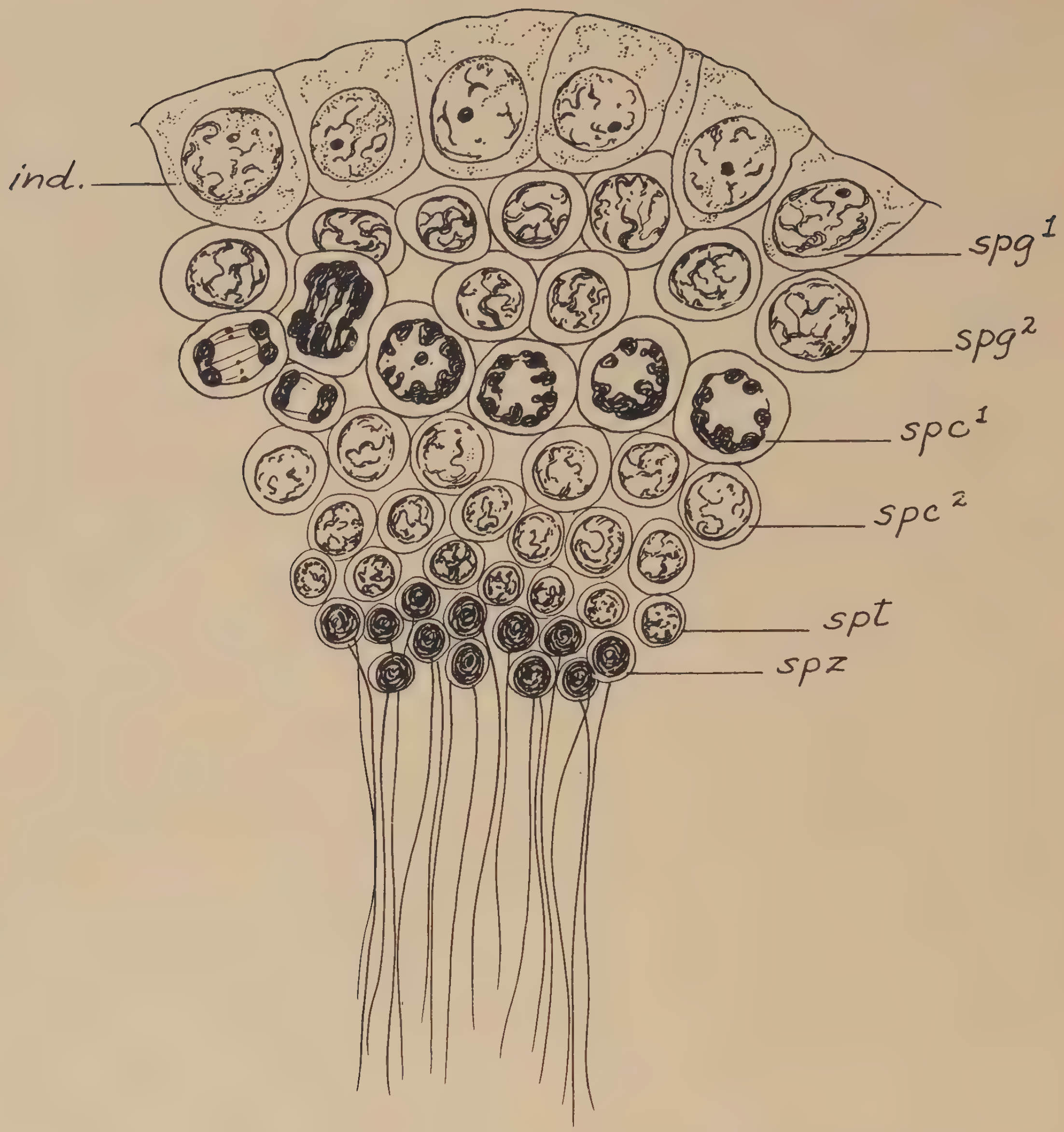

FIGURE 297.—Mature spermary of *C. virginica*. ind—indifferent cells; spg¹ and spg²—primary and secondary spermatocytes; spt—spermatids; spz—mature spermatozoa. Redrawn from Coe, 1932a, fig. 8.

secreted by the egg itself while it is still in the ovary. Raven (1958) states that in some cases the vitelline membranes of *Ostrea*, *Mytilus*, *Dreissensia*, and *Dentalium* are thrown off soon after shedding. I have not seen this happen in the eggs of *C. virginica*.

CYTOPLASMIC INCLUSIONS

Cytoplasmic components of an oyster egg are not well known primarily because the ultrastructure has not been studied by electron microscopy. Certain types of minute granules can be seen, however, in examination of live eggs under high magnification of phase contrast lenses; by applying vital and metachromatic stains; by centrifuging whole eggs or their homogenates in order to separate various components and study their staining reactions. For descriptions of the techniques used in modern cytology the reader is referred to the textbooks on cytology and microscopic histochemistry (Gomori, 1952; DeRobertis, Nowinski, and Saez, 1960; and others). The yolk constitutes the major part of the eggs of marine bivalves. Quantitative data on the amount of yolk in oyster eggs are lacking, but for *Cumingia tellinoides* and *Mytilus californianus* Costello (1939) found that

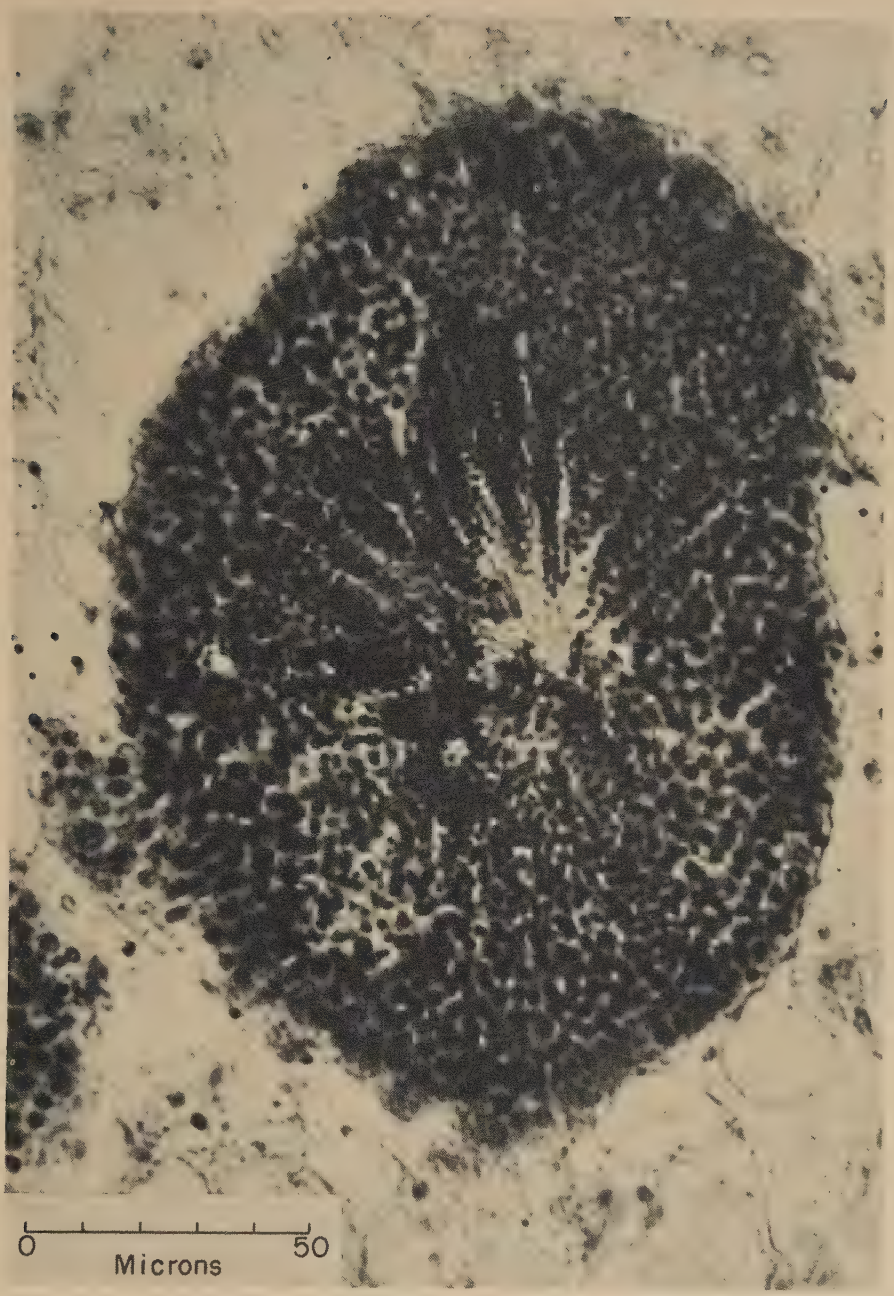

FIGURE 298.—Photomicrograph of a cross section of one follicle of a fully mature spermary of *C. virginica*. Hematoxylin-eosin.

yolk forms 35 and 31 percent respectively of the total egg volume. The estimates were obtained after a centrifugal force of 20,000 (*Cumingia*) and 4,800 (*Mytilus*) times gravity had been applied to the unfertilized eggs. In the cytoplasm of the eggs of the two species the relative volumes of hyaline zone were 42 and 55 percent and of the oil 10 and 14 percent.

The yolk of molluscan eggs is made of two types of granules, one of proteid and the other of fatty materials. In cytological literature the distinction between the proteid yolk and fatty yolk is not always made clear. In descriptions of the cytoplasmic inclusions of an egg based on light microscopy some authors apply the term exclusively to protein granules, while others, including Gatenby (1919), Gatenby and Woodger (1920), and Brambell (1924) in their studies of the

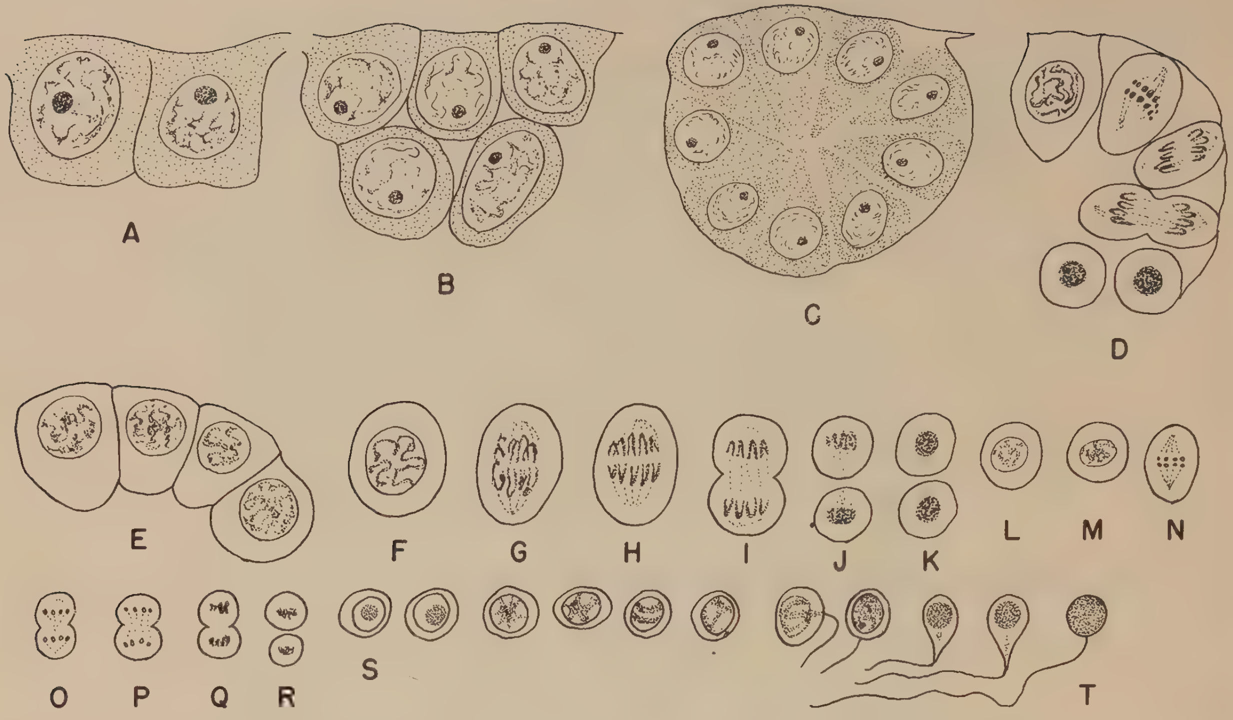

Figure 299.—Diagram of successive stages of spermatogenesis in *O. lurida*. A—two indifferent germ cells on the wall of the gonad; B—small group of spermatogonia, with reticular chromatin and conspicuous nucleoli; C—small group of secondary spermatogonia; D—division of secondary spermatogonia to form spermatocytes; E—primary spermatocytes with slender chromosomes; F to K—division of primary spermatocytes; L to R—division of secondary spermatocyte; S to T—transformation of spermatid into the mature spermatozoon. Redrawn from Coe, 1931, fig. 2.

gametogenesis in the gastropods *Helix*, *Limnaea*, and *Patella*, identify the protein granules as "mitochondria" and restrict the term yolk to fatty inclusions.

The role of the mitochondria in the formation of yolk has not been fully resolved. According to Rai (1930), the fatty yolk in the eggs of *C. cucullata* is formed directly from the Golgi vesicles as it is in ascidians, *Helix*, and other invertebrates; mitochondria do not participate in the vitellogenesis, and albuminous yolk is absent in the egg of the Indian oyster (*C. cucullata*). This view is in agreement with the conclusion of Worley (1944), who found no protein yolk in the eggs of *Mytilus* and *Ostrea*. The question is not settled because apparently the cytologists have no clear agreement on the difference between the protein yolk and mitochondria.

A study of cytoplasmic inclusions was made by Cleland (1947, 1951), who separated the granules found in egg cytoplasm of the oyster by differential centrifugation following homogenization. Mature eggs were suspended in a solution of 0.2 M potassium chloride and 0.02 N sodium citrate buffered to pH 7.5. Homogenates were obtained by blending the suspension in an electric blender surrounded by an ice jacket. By centrifuging the samples of homogenates at different speeds the following types of granules were obtained: P granules or protein yolk; L granules or lipid yolk; M granules or mitochondria; and S or submicroscopic granules. The P granules obtained by Cleland's technique are spherical and can be stained by Janus green B in the test tube. In the living egg these granules are located along the periphery and absorb Nile blue stain. After being centrifuged at 5,000 times gravity for 5 minutes they form a thick centrifugal layer with a sharp upper boundary. Alpha or lipid yolk granules are also spherical. They occupy the central part of the living egg. In the centrifuged egg they form a centripetal layer with a sharp lower boundary. Alpha granules of phospholipid and neutral fat can be recovered from the supernatants of homogenate suspensions. M granules or mitochondria in the live centrifuged egg form a thin, rather loose layer above the P granules and stain both with Janus green B and Nile blue.

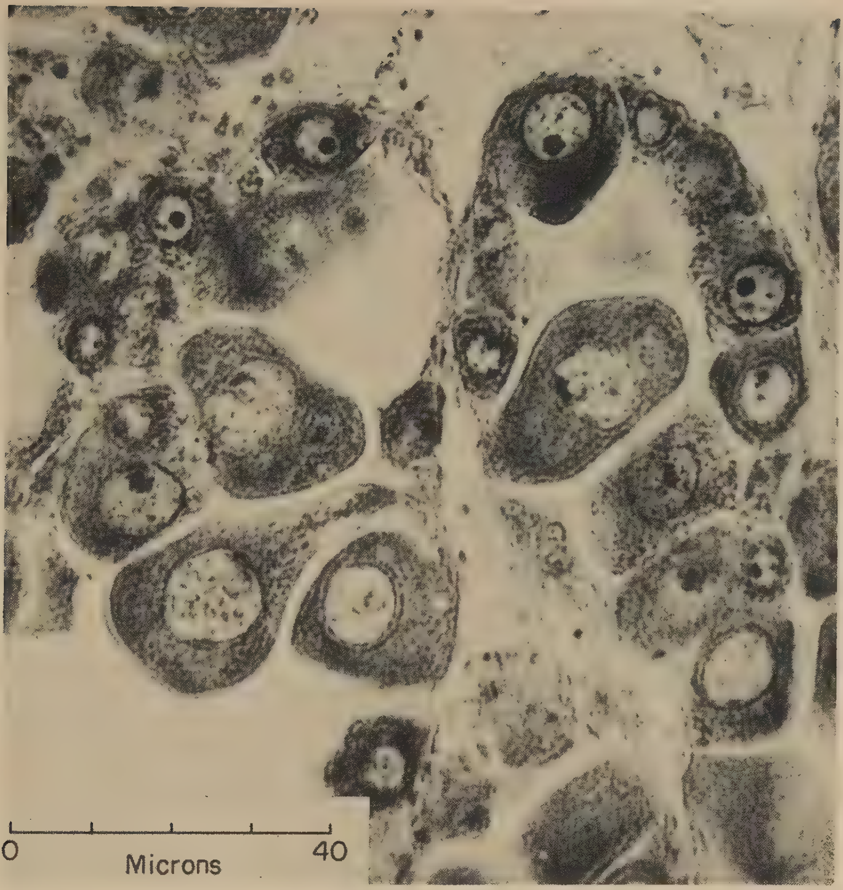

FIGURE 300.—Photomicrograph of eggs in the follicles of the ovary of *C. virginica* at the beginning of the spawning season. Ovocytes and small indifferent cells line the wall; mature eggs are either free or connected to the wall with long peduncles. Kahle, hematoxylin-eosin.

Cleland states that in the uncentrifuged mature egg they are unrecognizable. From homogenates the M granules can be separated by centrifuging for 10 minutes at 10,000 times gravity. Cytoplasm also contains submicroscopic or S granules (according to Cleland's terminology), which are separable by applying a centrifugal force of 20,000 times gravity for 30 minutes. These S granules are probably homologous to mammalian microsomes, i.e., the submicroscopic ribonucleoprotein particles which are considered to be the major sites of protein synthesis (a discussion of this problem is found in Shaver, 1957, and Novikoff, 1961b).

With the exception of pure lipid granules, the cytoplasmic components of the egg of *C. commercialis* show an increasing content of nucleic acid with decreasing size of granules, the ground cytoplasm containing the highest concentration of nucleic acid. Cleland's observations need to be corroborated, using the eggs of different species of oysters.

The formation and composition of yolk in the eggs of animals other than bivalves have been studied by many investigators, frequently with different and sometimes contradictory results. As Brachet (1944) stated nearly 20 years ago, the problem cannot be resolved at present. This uncertainty about yolk and other granules still persists and probably will continue until the ultrastructure of the marine egg is thoroughly explored by electron microscopy.

Examination with the light microscope of ripe, unfertilized, and unstained eggs of *C. virginica* discloses a multitude of tightly packed minute granules in the cytoplasm which obscure the inner portion of the egg. The granules appear to be uniformly distributed around the nucleus (fig.

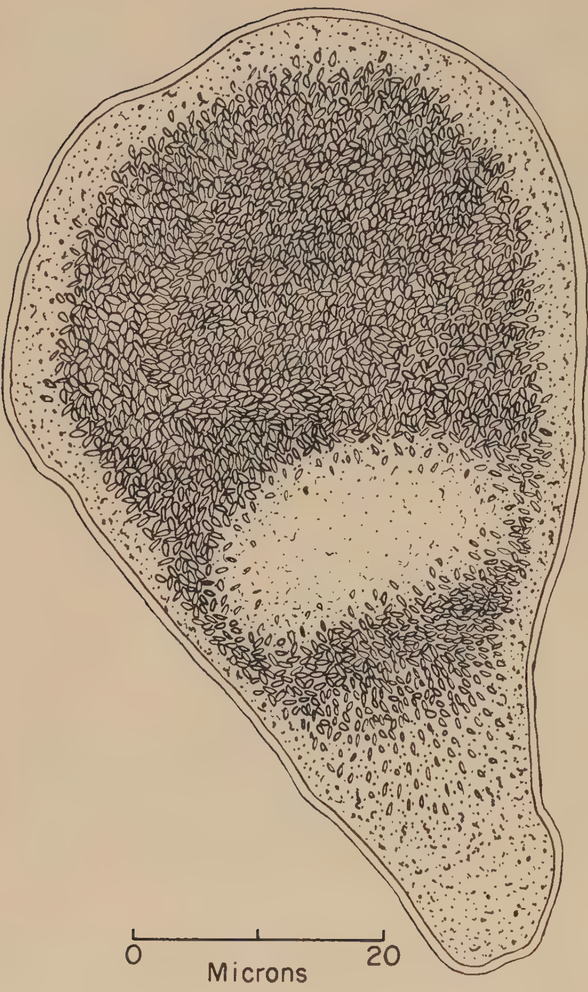

Figure 301.—Camera lucida drawing of live unfertilized egg of *C. virginica* in sea water. Germinal vesicle not visible under yolk granules.

301). The eggs are devoid of pigment. Oil globules of different sizes can be made visible under high magnification by gently pressing the egg under a coverslip; by using fat-staining dyes (Sudan II, III, or Black Sudan B) they become conspicuous (fig. 303). Under the effect of dye (dissolved in weak alcohol) the small granules of lipid yolk, stained dark red or black, gradually fuse into large globules and penetrate the vitelline membrane and a slight pressure will force them through it (fig. 304). The size of the globules increases during the time that the preparation remains under the microscope. These artifacts are due to the fusion of globules under the effect of dye.

The mitochondria of *C. virginica* can be stained by a 0.5 percent solution of Janus green in sea water. They appear as small rodlike structures uniformly distributed in the subcortical layer of the egg (fig. 305). The origin of fatty or lipid yolk in *C. virginica* has not been studied. In *Mytilus* eggs the lipid of the yolk apparently arises in an intimate association with the Golgi apparatus (Worley, 1944). In *Lymnaea* (Bretschneider and Raven, 1954) they are formed in certain parts of the protoplasm independently of cell structures visible under the light microscope.

In the eggs of the Bombay oyster, *C. cucullata*, which are similar to those of *C. virginica*, the fatty yolk, according to Rai (1930), is formed directly from the Golgi vesicles. Mitochondria exist in the eggs of this species in the form of very minute granules forming a circumnuclear ring. Later they grow in size and are more or less uniformly distributed. This conclusion is in agreement with the observations of Gatenby and Woodger (1920), who found that in *Helix* and *Limnaea* the Golgi elements gradually spread throughout the ovocyte and probably take part in the formation of yolk bodies. They found no evidence that part of the mitochondrial constituents of cytoplasm metamorphose into yolk.

Oyster eggs placed for 5 minutes in a dilute solution (1 to 25,000 or 1 to 30,000) of toluidin blue 0 and washed in sea water are colored metachromatically. Pasteels and Mulnard (1957) found

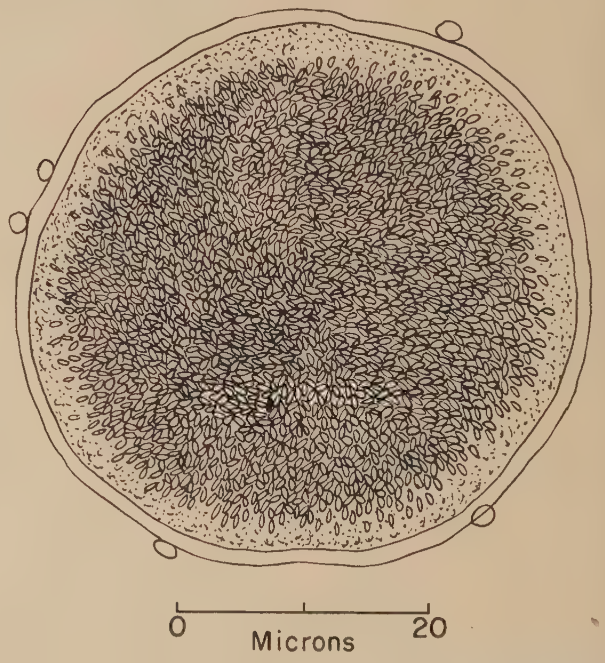

Figure 302.—Camera lucida drawing of live egg of *C. virginica* a few minutes after fertilization.

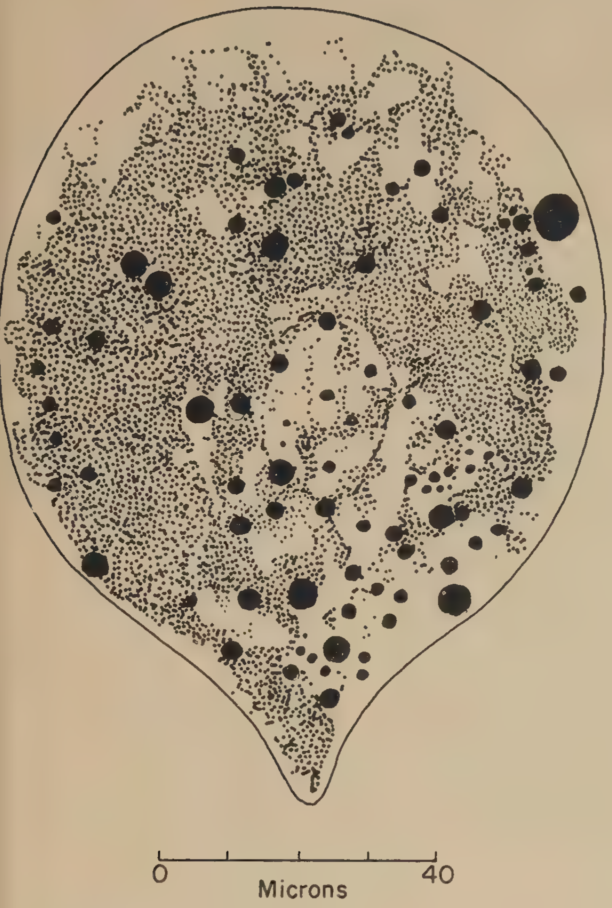

FIGURE 303.—Oil globules in the unfertilized egg of *C. virginica* after staining in Sudan III. Whole mount. Drawing made from a photomicrograph.

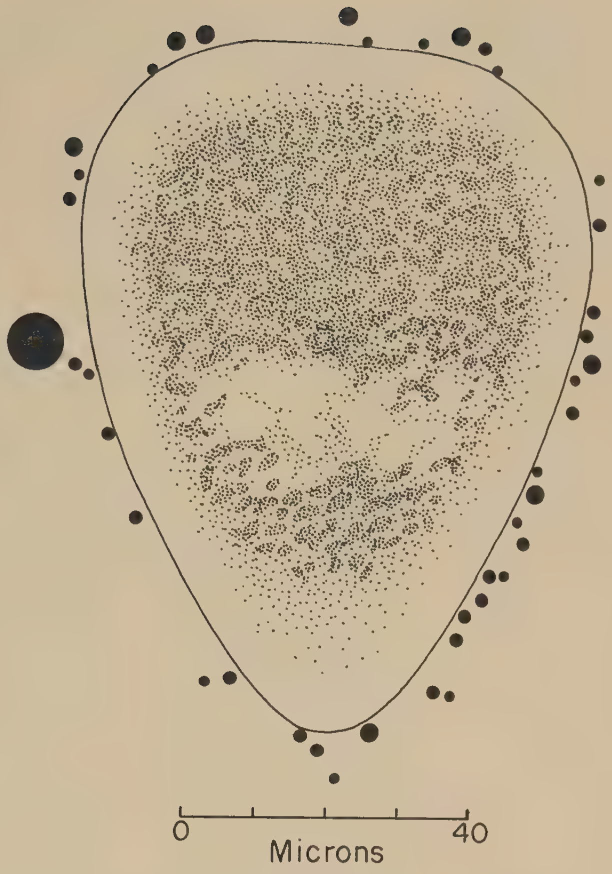

FIGURE 304.—Unfertilized egg of *C. virginica* stained with Black Sudan B; slight pressure on a coverslip forces the oil globules through the vitalline membrane. Drawn from life.

that the development of eggs of the Portuguese oyster, *C. angulata*, is not affected by toluidin blue used in such dilute solution for only a short time. The dye is fixed at the level of the small granules, which the cytologists designate as alpha granules, uniformly distributed in the cytoplasm between the yolk vesicles. Later in the development of a fertilized egg, new and larger granules, called beta granules, appear at the time of prophase. Their higher metachromasy is acquired at the expense of the alpha granules. Subsequent studies (Mulnard, Auclair, and Marsland, 1959) have suggested that the beta granules are related to the Golgi complexes of the eggs.

The alpha granules of the unfertilized eggs of *C. angulata* can be separated from the yolk vesicles by centrifuging; they are displaced in the direction of the centrifugal force (Pasteels and Mulnard, 1957), while the beta granules at the pronucleus stage of the fertilized egg are moved in the centripetal direction. The alpha and beta particles of Pasteels and Mulnard probably correspond to the P and L granules of Cleland. Personal observations show that in ripe but unfertilized eggs of New England *C. virginica* stained with toluidin blue, elements corresponding to the alpha particles of Pasteels assume a lavender color while mitochondria and other smaller granules are bluish. The nucleolus is also of bluish color. After 10 minutes of centrifuging at 4,000 times gravity the yolk granules of the stained eggs concentrate at the lower (centrifugal) pole, while the alpha particles of lavender hue and slightly bluish mitochondria are at the opposite pole (fig. 306).

Metachromatic granules have been described in the eggs of various bivalves. They were found in *Barnea candida* (Pasteels and Mulnard, 1957); *Mactra* (Kostanecki, 1904, 1908; *Mercenaria* (*Venus*) *mercenaria*, *Mytilus edulis*, and *Spisula solidissima* (Worley, 1944; Kelly, 1954, 1956;

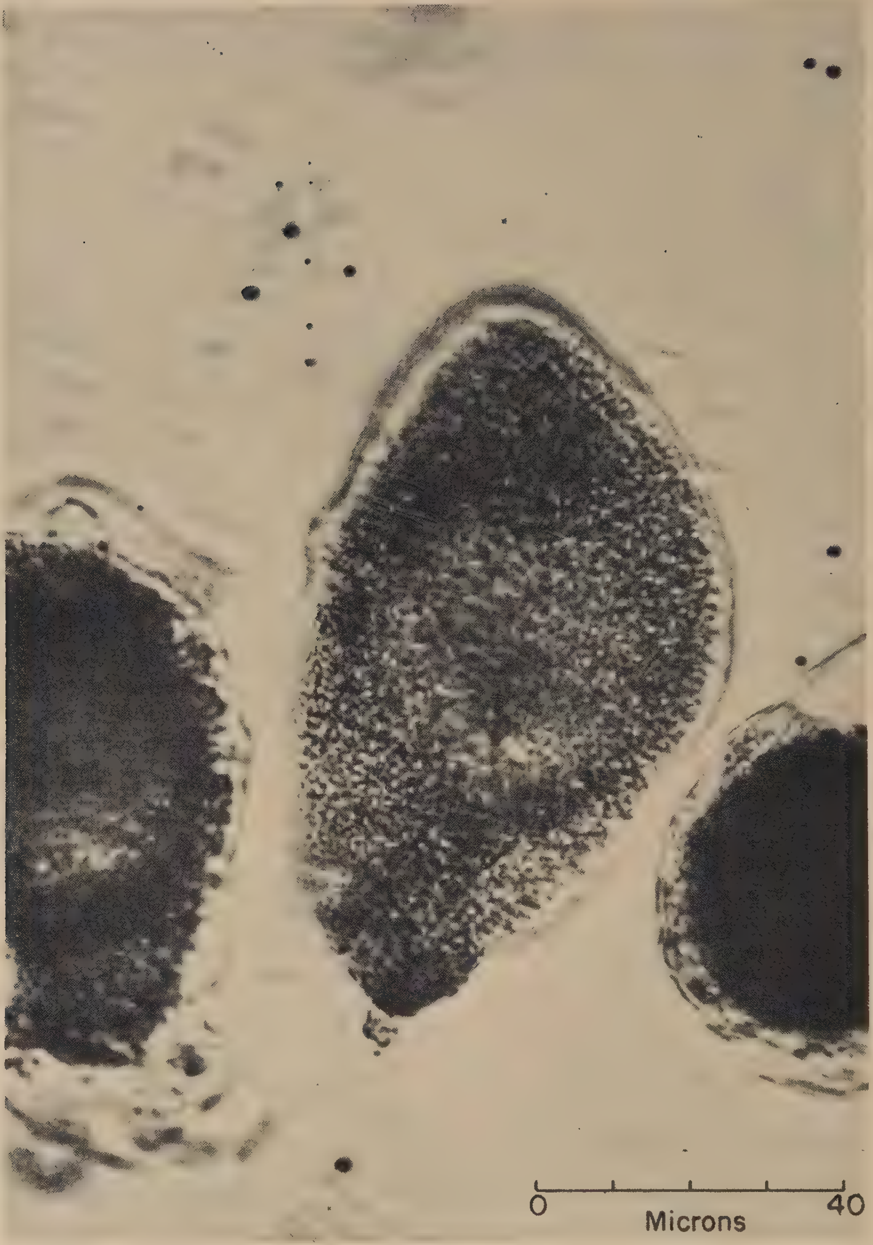

FIGURE 305.—Photomicrograph of an unfertilized egg of *C. virginica.* Janus green. Whole mount. Small rodlike inclusions seen along the periphery are mitochondria stained green; larger yolk granules are dark.

Allen, 1953; and Rebhun, 1960). The role of these almost submicroscopical bodies is not clear, but there is no doubt of their importance in the physiology and development of eggs. Recent publications of Dalcq (1960), Brachet (1960), and Rebhun (1960), and the reviews given by Novikoff (1961a, 1961b) should be consulted for ideas concerning the possible role of these elements in the morphogenesis of mosaic eggs in which they are concentrated in the posterior blastomeres.

Eggs of the surf clam *S. solidissima* contain a heparinlike blood anticoagulant which was also extracted from the tissues of this clam (Thomas, 1954). Whether substances with similar activity are present in oyster eggs is not known.

The nucleus of a mature egg is surrounded by a nuclear membrane which can be clearly seen on sectioned and stained preparations of the ovary (fig. 300). A spherical, dense nucleolus is excentrically located; its diameter varies from 4 μ to 6 μ.

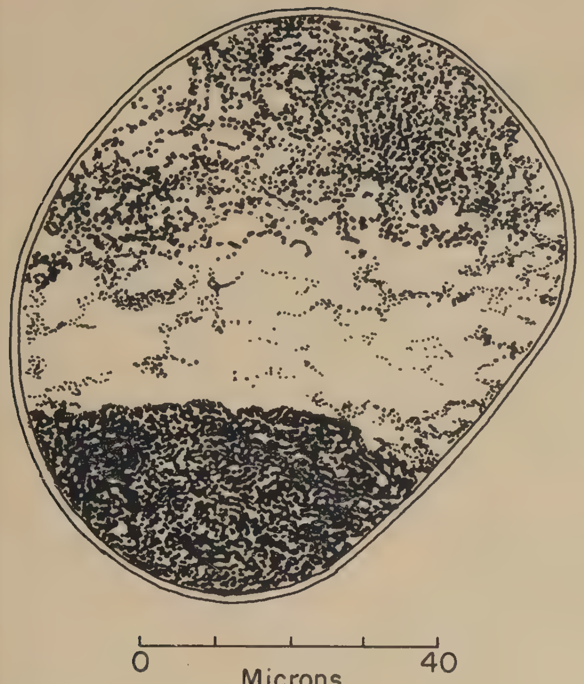

FIGURE 306.—Unfertilized egg of *C. virginica* centrifuged for 10 minutes at 4,000 times gravity after staining with toluidin blue. Dark yolk granules are at the lower (centrifugal) pole while the mass of small inclusions consisting of lavender particles and bluish mitochondria are at the opposite end of the egg.

The chromosomes appear as fine threadlike structures near the nuclear membrane. Using methyl green and pyronin B stains, Kobayashi (1959) found that the nucleolus in the eggs of *C. gigas* and *O. laperousi* consists of two parts, the karyosome, shown in solid black in fig. 307, and the plasmosome, lightly shaded, in close contact with each other. The definite KP axis (Karyosome-Plasmosome) of the unfertilized egg changes after fertilization by the turning of the karyosome around the plasmosome. The existence of this axis in the eggs of other species of oysters has not been described.

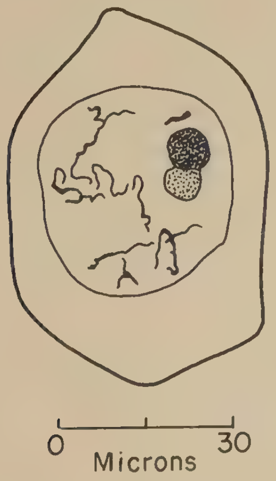

FIGURE 307.—Ovocyte of *O. laperousi* stained with methyl green and pyronin B after Navashin fixation. Dark globule is the karyosome, light shaded area is the plasmosome. From Kobayashi, 1959.

STRUCTURE OF THE MATURE SPERMATOZOON

The spermatozoon of bivalve mullusks appears under the microscope (Franzén, 1956; Lenhossék, 1898; Retzius, 1904) to consist of an oval or round head with a pointed front, a middle piece at the lower end of the head, and a long tail (flagellum) with a narrow "end piece" which is longer than the width of the head. The middle piece consists of four, sometimes five, oval-shaped bodies clustered around the tail; and a minute "central granule" or centriole located in the center at the point of attachment of the tail. The sharply outlined oval bodies are mitochondria; they are strongly osmiophilic and can be deeply stained with rosanilin. The head of the spermatozoon is formed by a compact nucleus capped with the apical body or acrosome with a pointed tip (perforatorium), which apparently assists the spermatozoa in penetrating the egg membrane at fertilization (Wilson, 1928). The features listed above may be seen in properly fixed and stained preparations of the sperm of *C. virginica* and in live spermatozoa examined with phase contrast oil immersion lenses. In live preparations the nucleus appears to be dark while the acrosome and middle piece are light (fig. 308). The center of the spermatozoon head is occupied by an axial body, a relatively large, light-refracting structure which is separated from the acrosome.

The dimensions of normal, uncytolyzed spermatozoa have been measured by means of an eyepiece micrometer of a light microscope. The head varies from 1.9 μ to 3.6 μ in length (median value 2.7 μ) and between 1.0 μ and 2 μ in width. The tail is from 27 μ to 39 μ long (median value 36 μ). The tails are usually slightly curved; specimens with straight tails are rarely found.

Electron microscopy reveals much greater complexity in the structure of the spermatozoon (Galtsoff and Philpott, 1960). Study was made of small sections of ripe spermary preserved in cold 1 percent osmium tetroxide buffered to pH

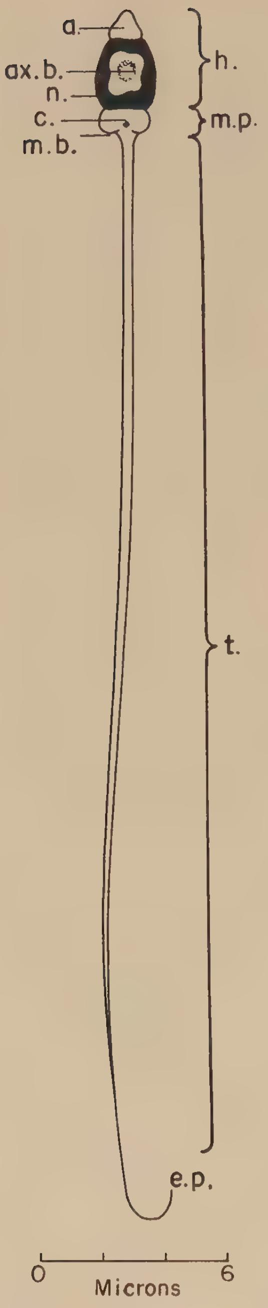

FIGURE 308.—Live spermatozoon of *C. Virginica* examined under light microscope with phase contrast oil immersion lens. A—acrosome; ax. b.—axial body; C—centriole; e.p.—end piece of tail; h.—head; m.p.—middle piece; mt.—mitochondrial bodies; N—nucleus; t.—tail.

7.2–7.4 and embedded in a mixture of three parts butyl and one part methyl methacrylate. To increase the contrast, some of the material was placed in 1 percent alcoholic phosphotungstic acid for 5 to 6 hours before embedding. The embedded material was sectioned on Philpott's microtome (Philpott, 1955), using a diamond knife. Since the preserved pieces consisted of a multitude of spermatozoa arranged in a central mass with their tails pointing outward, the individual spermatozoa were always cut at random in different planes, regardless of how the embedded tissue may have been oriented on a microtome block. The resulting electron micrographs showed a number of sperm heads cut at different planes and many transverse sections of tails (fig. 309). The entire structure of the head was diagrammatically reconstructed by bringing various elements together and placing them in their relative positions (fig. 310). The oval-shaped head consists of slightly granular, homogeneous material covered with an osmiophilic membrane made of two layers. The apical portion of the nucleus is occupied by a caplike acrosome of highly osmiophilic substance. The acrosome is clearly separated from the nucleus by a sharply defined membrane. An egg-shaped body in the central part of the nucleus extends from the apex of the acrosome almost to the base of the nucleus. This structure, named axial body (Galtsoff and Philpott, 1960), has a central stem of fibrous material which emerges from the flattened bottom and extends about two-thirds of the total length of the axial body. The indented base of the nucleus is near the base of the axial body. The caved-in space formed by this indentation consists of material of lesser electron density and extends under the nucleus to the upper surface of the centriole, which is surrounded by four mitochondrial bodies. Only two of them are shown in fig. 310.

The centriole of the sperm of *C. virginica* is a hollow, cylindrical structure with walls made of nine bands; these can be seen in cross section (fig. 311). The side view (fig. 310) shows that the centriole is formed in several alternating and slightly constricted layers which connect with the four mitochondrial bodies. These bodies have the typical appearance of twisted lamallae enclosed in a membrane which encompasses the centriole and continues over the tail.

The tail consists of a pair of axial filaments surrounded by a ring of nine double filaments spaced at equal intervals along the periphery (fig. 312). The filaments are interconnected by delicate strands. The axial filaments begin near the basal plate (fig. 310) where the tail emerges. Radial trabeculae connect the ring filaments to the outside wall of the tail and form nine separate

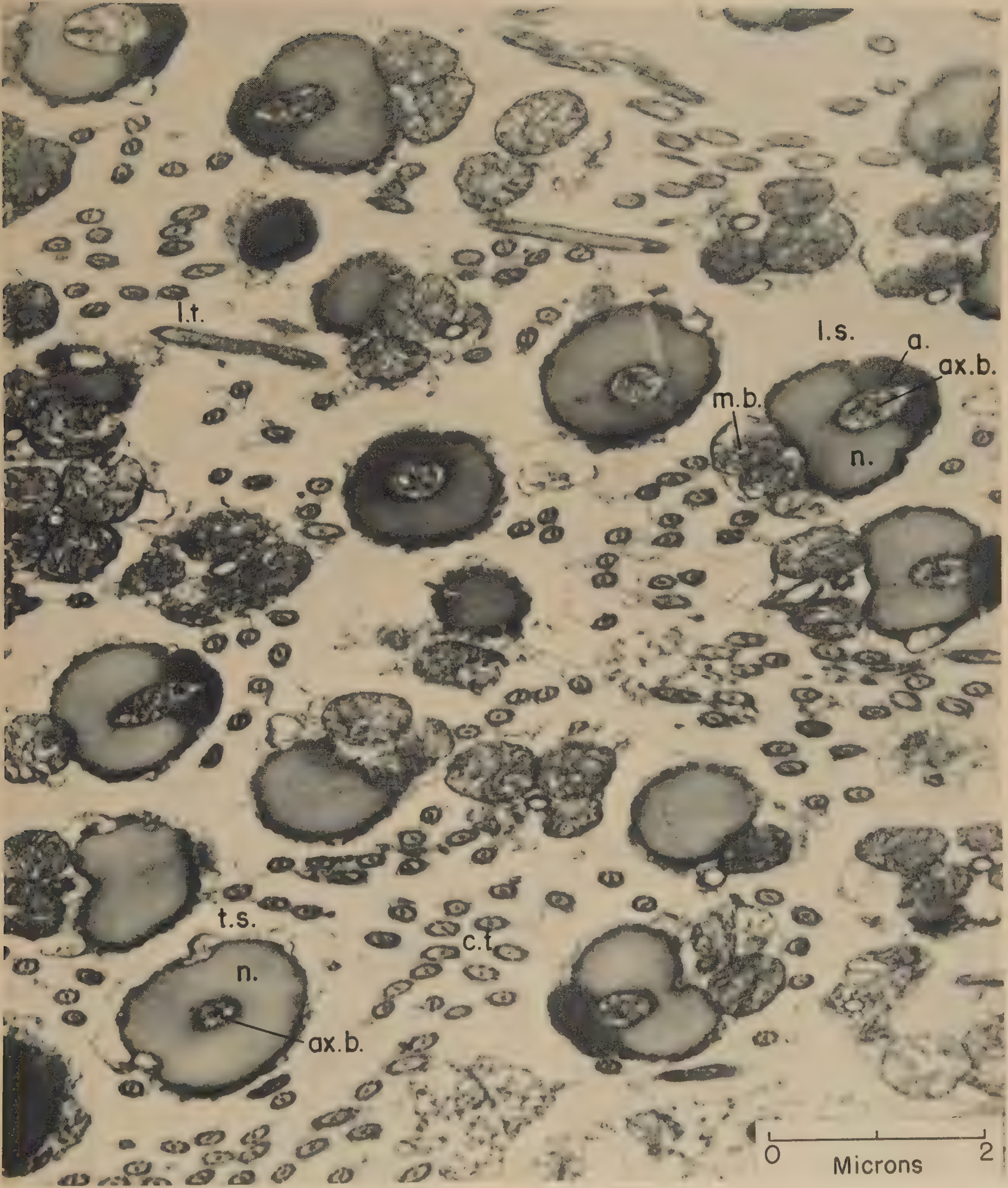

FIGURE 309.—Electron micrograph of a section of ripe spermary of *C. virginica*. Longitudinal and transverse sections of sperm heads and tails can be seen in various parts of the micrograph. A—acrosome; ax. b.—axial body; c.t.—cross section of tails; l.s.—longitudinal section of sperm head; l.t.—longitudinal section of part of a tail; m.b.—mitochondrial bodies; N—nucleus; t.s.—transverse section of head.

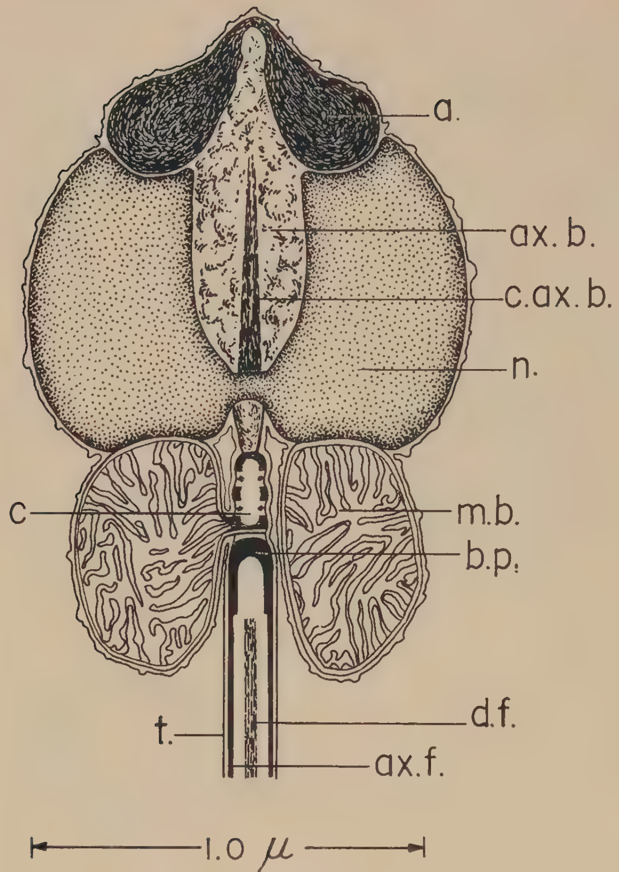

FIGURE 310.—Reconstruction of the sperm head of *C. virginica* made from a large number of longitudinal sections. a.—acrosome; ax. b.—axial body; ax. f.—axial filament of the tail; b.p.—basal plate of the tail; c.—centriole; c. ax. b.—core of the axial body; d.f.—double filament on the periphery of the tail; m.b.—mitochondrial body; n.—nucleus; t.—proximal part of the tail.

compartments filled with material of lesser electron density. The central radial strands are similar to the "spokes" described by Afzelius (1955) for the sperm of the sea urchin *Psammechinus miliaris*. They are not present in the proximal portion of the tail where there are no axial filaments, but otherwise the ultrastructure of the sperm tail is similar to that of cilia and flagella of various animals and plants.

FERTILIZATION

The spawned eggs of *C. virginica* and *C. gigas* are heavier than water and quickly sink to the bottom. The time they remain in suspension may be prolonged by horizontal currents and upward movements of the water, and consequently the chances of fertilization are increased. Because spawning is usually initiated by the males, the water into which the eggs are discharged already contains active spermatozoa and fertilization takes place within a few minutes following ovulation. It is obvious that the success of reproduction of an oyster population in which spawning is mutually stimulated by the discharge of sex cells is dependent on close proximity of the sexes and their simultaneous response to spawning stimuli.

Eggs and sperm secrete substances called gamones which play an important role in fertilization. Secretion from an unfertilized egg has a significant effect on spermatozoa. This effect can be observed if a suspension of eggs is permitted to stand for 15 to 20 minutes and the supernatant fluid is decanted or filtered and added to the suspension of sperm. The resulting so-called "egg water" (Lillie, 1919) causes the agglutination of sperm. To observe the agglutination reaction with the naked eye, a drop of egg water must be added to a sperm suspension, which shortly forms irregular lumps (fig. 313). Under a high-power light microscope one sees that the heads of agglutinated spermatozoa stick together to form large aggregates (fig. 314).

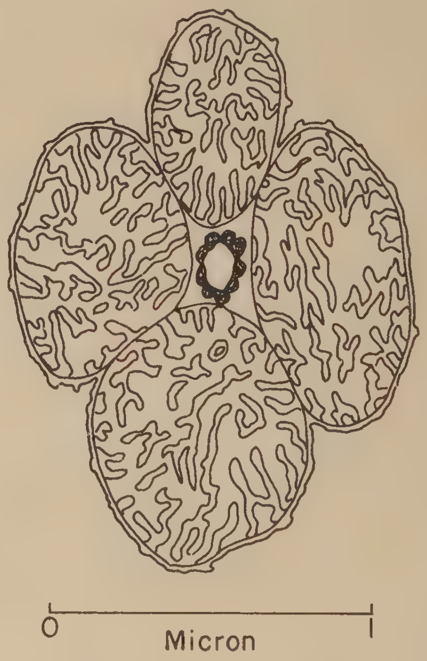

FIGURE 311.—Drawing based on electron micrographs of the cross section of the lower part of the middle piece of the spermatozoon of *C. virginica*. Centriole, at the center, is surrounded by four mitochondrial bodies.

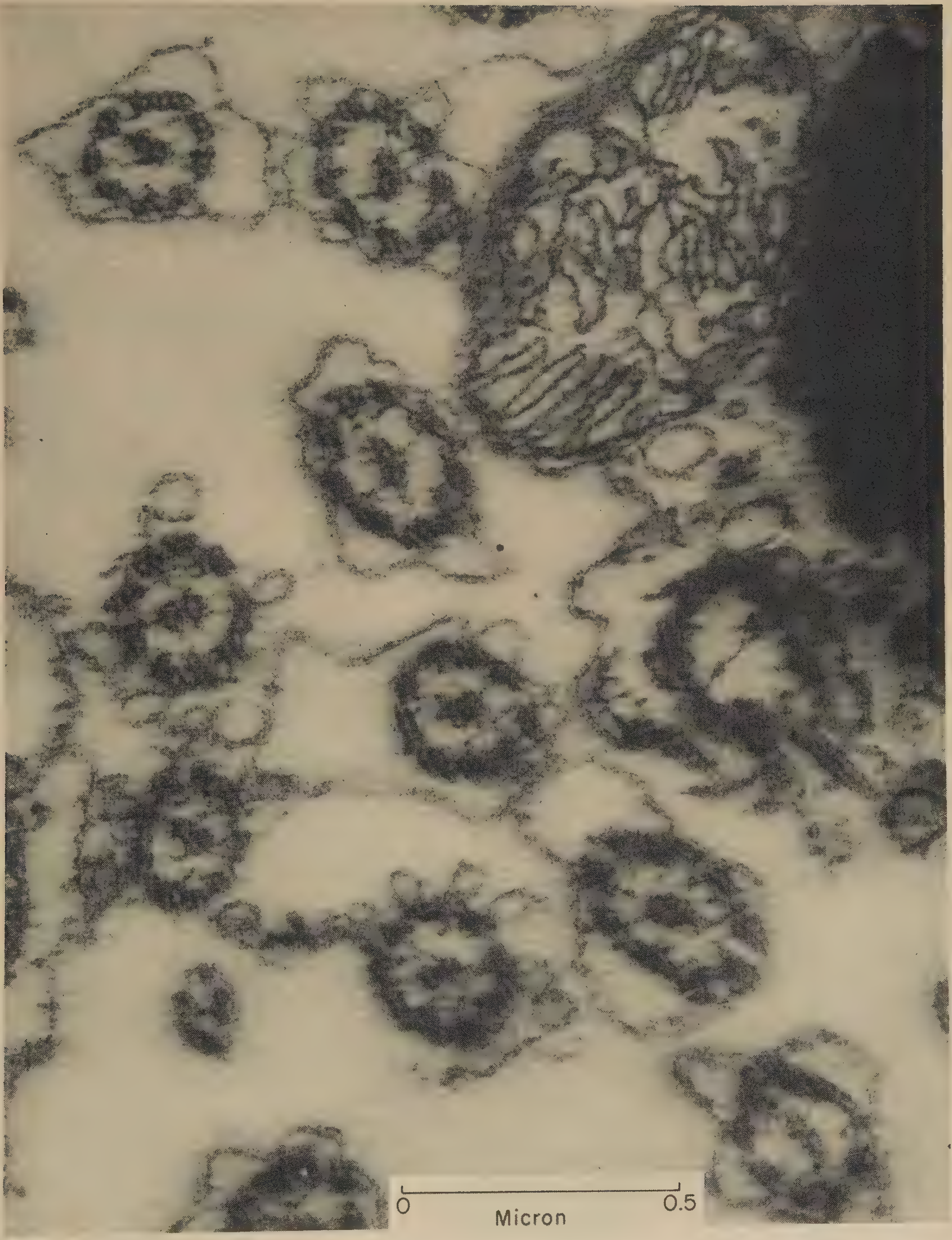

FIGURE 312.—Transverse section of the tails of oyster sperm of *C. virginica* slightly below the level of the middle piece. Electron micrograph.

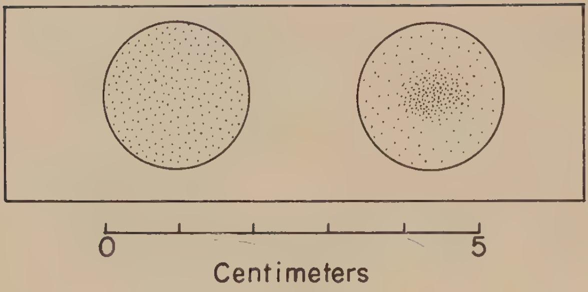

FIGURE 313.—Sperm suspension of *C. virginica* on a slide in sea water (left) and in sea water containing a small quantity of egg water (right). Drawn from life. Natural size.

Agglutination also occurs in the sperm of *C. gigas* and in *O. circumpicta* Pilsbry. Terao (1927) experimented with the latter species using egg water made by mixing 0.55 ml. of ripe eggs in 9 ml. of sea water and removing the eggs by centrifuging and filtering after they had stood for 20 minutes. The filtrate caused the isoagglutination of sperm even in a dilution of 1 to 10 millions. Heteroagglutination by the egg water of *O. circumpicta* has been observed in the suspensions of sperm of the bivalve *Arca*, sea urchin *Toxocidaris tuberculatus*, and starfish *Luidia quinaria*.

Lillie (1919) regarded the sperm agglutinating factor he discovered in *Arbacia* eggs as an essential to fertilization, and to the active substance of egg water he gave the name fertilizin. Tyler (1948) identified fertilizin with the jelly substance of the egg and on the basis of experimental data concluded that the presence of the jelly coat has a favorable effect on fertilization. By biochemical analysis of sea urchin eggs, Vasseur (1948a, 1948b) determined the composition of the jelly coat and found that 80 percent of it consists of polysaccharide and 20 percent of amino acids. The substance was found to exert a heparinlike action in a blood-clotting system (Immers and Vasseur, 1949). After removing the jelly coat with acidified water, Hagström (1956a, 1956b, 1956c, 1956d) found that the rate of fertilization was higher than in the presence of the coat. It is, therefore, apparent that the jelly coat is not essential for fertilization.

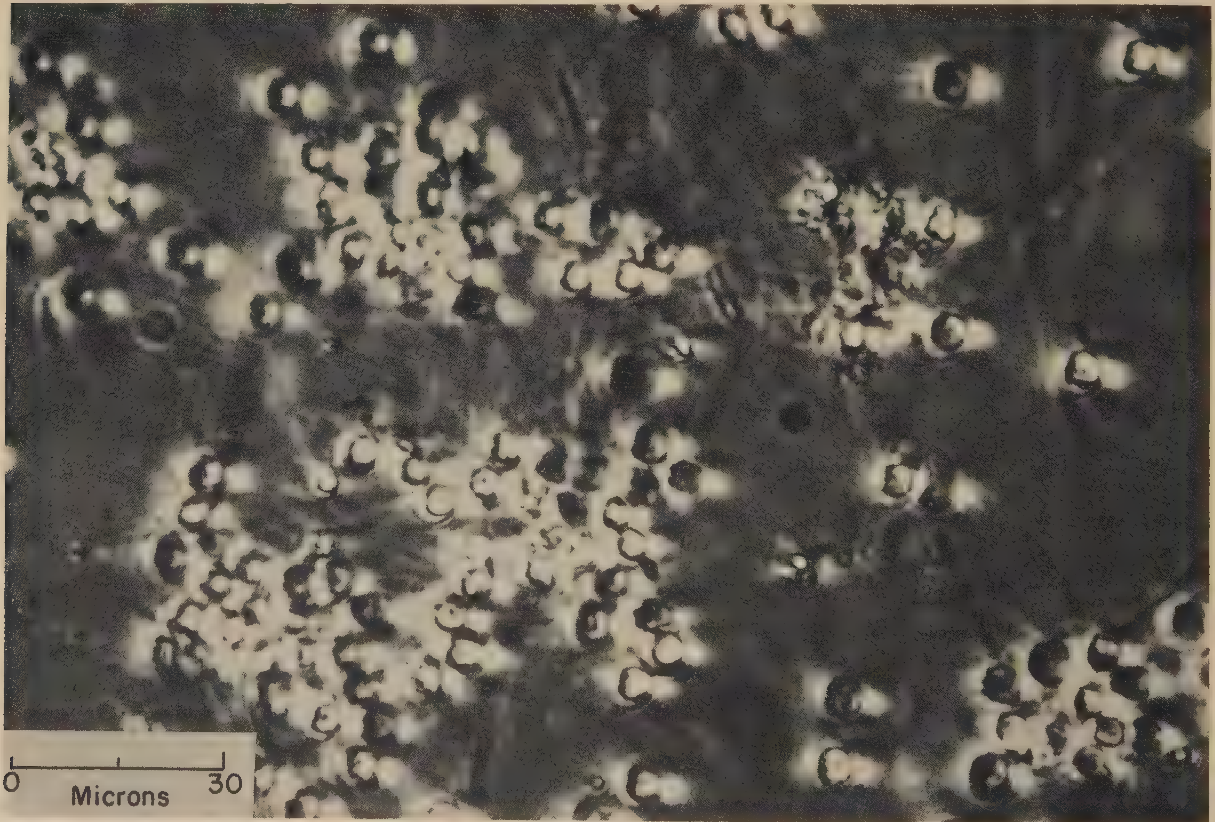

FIGURE 314.—Photomicrograph of sperm of *C. virginica* agglutinated by egg water of the same species. Phase contrast oil immersion.

This view agrees with the conditions found in oyster eggs which have no jelly coat.

According to the modern view discussed in the review of the problem by Runnström, Hagström, and Perlmann (1959), the jelly coat not only fails to improve fertilization but impedes it by acting as a sieve. Its action may be considered as an elimination process by which the number of spermatozoa capable of attaching to the cytoplasmic surface is substantially reduced.

Fertilizin of sea urchin eggs has two distinct properties: it agglutinates sperm suspension and activates the motility of free, single spermatozoa. Both of these properties are present in the fertilizin of an oyster egg.

ACROSOMAL REACTION

Spermatozoa of various invertebrates have been found to carry a substance of protein character, probably a lysine, capable of dissolving the vitelline membrane of the egg. Such lysine is present in the sperm of the giant keyhole limpet, *Megathura crenulata* (Tyler, 1939), in the sperm of *Mytilus*, where it is probably located in the acrosome (Berg, 1950; Wada, Collier, and Dan, 1956), and in other marine animals (Tyler, 1948, 1949).

Upon contact with the surface of an egg, the spermatozoon undergoes a so-called acrosomal reaction, which is described as the deterioration of the surface of the acrosomal region of the head followed by a projection of a stalklike filament. The acrosomal reaction and the discharge of the filament have been observed in starfish, holothurians, mollusks, and annelids. The reaction was studied by Colwin, A. L., and L. H. Colwin (1955) and Colwin, L. H., and A. L. Colwin (1956) in the annelid *Hydroides hexagonus* and enteropneust *Saccoglossus kowalewskii*. Using electron microscopy, the Colwins revealed many interesting details of the penetration of the spermatozoon into egg cytoplasm. In pelecypod mollusks the discharge of the acrosomal filament was observed in *Mytilus* and in the three species of oysters, *C. echinata*, *C. nippona*, and *C. gigas* (Wada, Collier, and Dan, 1956; Dan and Wada, 1955). The reaction can be induced by egg water as well as by the contact of a spermatozoon with the egg surface. The first sign of acrosomal reaction in oyster sperm is the flattening of the anterior surface of the spermatozoon. At the same time the head becomes extremely adhesive, the acrosome membrane bursts, and the filament is discharged. The reaction can be observed when a small drop of live sperm suspension is placed on a cover slip, a minute quantity of egg water is added, and the cover slip then inverted on a slide. The preparation is examined with phase contrast oil immersion lens using anisol (Crown oil) of refractive index 1.515 instead of cedar oil.

The acrosome reaction of *C. virginica* is similar to that observed by Dan and Wada in three other species of oysters. Under the effect of egg water the head becomes swollen and rounded and the filament is ejected from the acrosome. The discharged filament is wider than the tail and is about three to four times longer than the length of the head (fig. 315). In my observations only a small number of oyster spermatozoa suspended in egg water discharged acrosomal filaments.

The exact role of the filament in the fertilization of oyster eggs is still unknown. Investigations

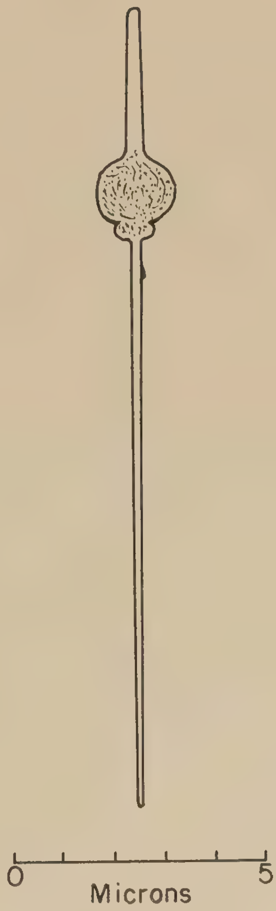

FIGURE 315.—Diagrammatic drawing of acrosomal reaction in the spermatozoon of *C. virginica* produced by egg water. Only a part of the sperm tail is shown in the drawing. Drawn from live preparation.

with eggs of other invertebrates suggest that the acrosome region of a spermatozoon is active during the first stages of fertilization and that it carries a lysine which facilitates the attachment of the spermatozoon to the egg membrane and its penetration into the cytoplasm.

The old view that spermatozoa penetrate the egg by a mechanical action of screw-borer movements of the pointed end (the perforatorium) has been abandoned. It is now generally accepted that the action of the sperm head is primarily chemical and that probably several enzymes are carried by the acrosome. Readers interested in the problem of fertilization are referred to comprehensive reviews of this subject by Runnström, Hagström, and Perlmann (1959), Colwin, A. L., and L. H. Colwin (1961a, 1961b), and Colwin, L. H., and A. L. Colwin (1961).

FERTILIZATION OF EGG

Eggs for fertilization experiments may be obtained in the laboratory by stimulating a single female spawn as described in chapter XIV. A suspension of eggs pipetted off the bottom of a laboratory tank is free of blood and other body fluids. Eggs may also be taken directly from the ovary by cutting off small slices from the surface of the gonad and mincing or shaking them in sea water. Cutting into the underlying layer of digestive diverticula should be avoided to prevent contamination with body fluids. The eggs must be washed several times in filtered seawater by decanting or by filtration through a fine sieve until the suspension is free of tissue cells and debris. After being in sea water for a short time, the eggs change their shape and become globular but their large germinal vesicles remain clearly visible (fig. 316).

A sperm suspension may be obtained by any one of three methods. Male spawning can be induced by raising the water temperature or by adding a small amount of thyroid suspension, and live spermatozoa collected as they are discharged through the cloaca; small pieces of ripe spermary can be excised and the spermatozoa liberated in sea water by shaking; or a very ripe spermary can be pressed gently with the fingertip and the spermatic fluid pipetted as it comes from the gonoduct. Concentrated sperm suspension must be diluted for fertilization. I found it convenient to make a standard suspension using 0.2 g. of gonad material in 50 ml. of sea water and then diluting it, using 0.5 ml. for 100 or 150 ml. of water containing eggs.

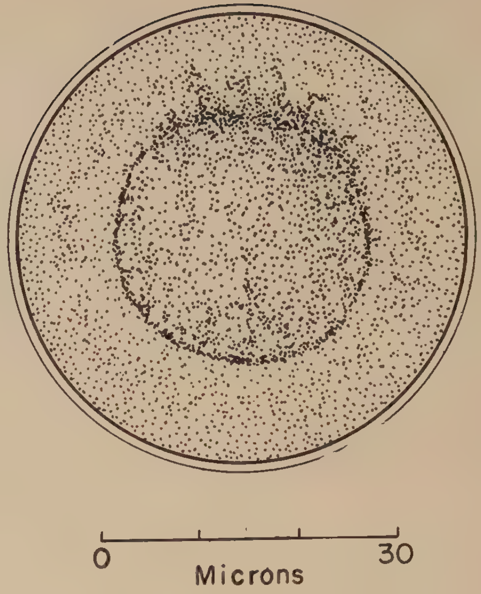

FIGURE 316.—Camera lucida drawing of naturally spawned but unfertilized egg of *C. virginica.*

Although several spermatozoa may attach themselves to an egg, (fig. 317), only one penetrates the cytoplasm. The others, called supernumeraries, eventually are cast off when cleavage

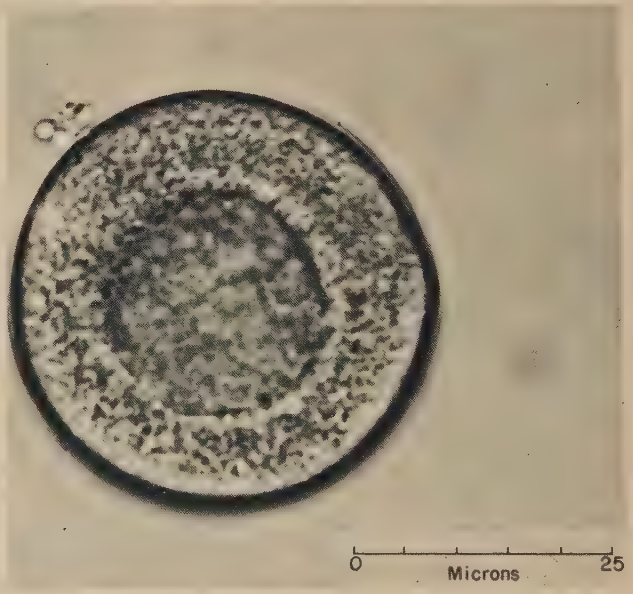

FIGURE 317.—Photomicrograph of a fertilized egg of *C. virginica* a few minutes after the formation of the fertilization membrane. Several spermatozoa are attached to the egg membrane but only one will penetrate it. The germinal vesicle is intact. Contrast phase oil immersion lens.

begins. If the sperm suspension is too concentrated, many spermatozoa enter one egg and cause polyspermy, a condition which may interfere with normal development of the egg.

The spermatozoon which succeeds in penetrating the egg's surface undergoes great changes. Its acrosome region becomes swollen and disrupted and the tail loses its motility; the head gradually penetrates the egg membrane as the sperm moves deeper into the cytoplasm. At the same time the fertilized egg contracts and assumes a globular shape if it was not round before; the cytoplasm becomes so dense that the germinal vesicle is no longer visible under the layer of yolk granules. A few seconds after the sperm head touches the egg's surface a thin, transparent fertilization membrane is elevated and under the light microscope appears to be homogeneous. This membrane apparently is formed from the pre-existing vitelline membrane and is underlined by a layer of subcortical particles (fig. 318). The two layers are optically separated. It is generally accepted (Runnström, 1952) that in *Arbacia* and many other species the fertilization membrane originates from the vitelline membrane because it fails to form after the vitelline membrane has been removed with potassium chloride, trypsin, or urea. No experimental work of this type has been done on oyster eggs.

AGING OF EGGS AND SPERM

The longevity of eggs of marine invertebrates, i.e., their ability to form fertilization membrane and undergo cleavage, was observed in the sea urchin (*Arbacia*) and in other common species (Harvey, 1956). Oyster eggs also undergo aging changes and lose their ability to be fertilized. This has been demonstrated in a number of tests made in the Bureau's shellfish laboratory at Woods Hole. Because of wide individual variability in fertilization capacities only one female and one male were used in each series of tests. The following technique was used: Suspension of eggs was made by shaking 0.5 g. of ripe ovary tissue in about 200 ml. of sea water: eggs released by this action were permitted to settle on the bottom and the supernatant water was decanted; the remaining eggs were rinsed twice in sea water and transferred to a beaker filled with 500 ml. of filtered sea water. The beaker was kept half submerged in running sea water to prevent heating to room temperature. Samples of eggs were taken for fertilization every hour during the first 4 hours, then at 2-hour intervals for the next 6 hours, and finally one sample was taken each time after 12 and 24 hours. Eggs were collected at random from the bottom of the beaker and placed in a finger bowl in 100 ml. of filtered sea water. To fertilize them 0.5 ml. of dilute stock suspension of sperm was used; the water was gently stirred

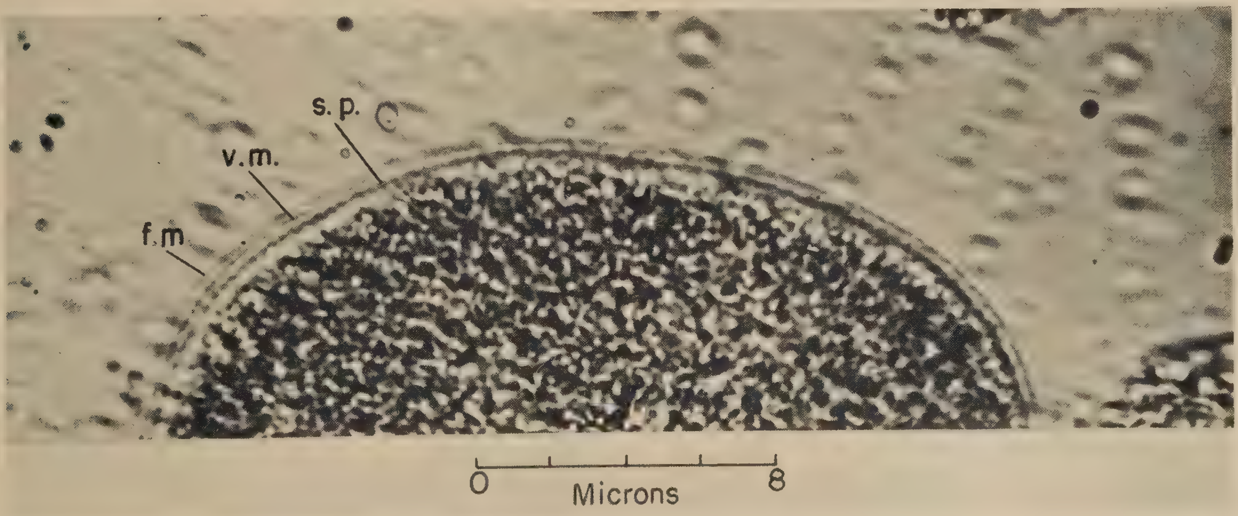

FIGURE 318.—Photomicrograph of a portion of fertilized egg of *C. virginica* shorly after the attachment of sperm. Fertilization membrane (f.m.) (outside layer) is underlined by the vitelline membrane (v.m.) with a dense row of subcortical particles (s.p.). Live preparation. Oil immersion phase contrast lens.

to obtain uniform distribution of sperm, and the finger bowl set aside for 5 hours, half submerged in running sea water. At the expiration of this period a sample was taken for examination and cleaved and uncleaved eggs were counted. In each case 300 eggs of the sample were examined. All tests were made in water of 31 to 32°/₀₀ salinity and 20.8° to 21.4° C. During the first 4 hours of aging the percentages of cleaved eggs declined from 90 to 70. After 5 or 6 hours the percentages dropped to 60. Then the fertilizability decreased to about 20 percent in 10 hours, and only a few eggs cleaved normally after 12 and 24 hours of aging.

It is common knowledge among embryologists that the fertilizing power of spermatozoa is not decreased if sperm is kept at 10° to 12° C. in a concentrated suspension in a tightly closed container. This is also true for oyster sperm. Its fertilizing power is affected by dilution and increased temperature. At room temperature in a dilute suspension, the spermatozoa lose their fertilizing ability within 4 to 5 hours. However, in a concentrated suspension, protected from evaporation, and stored in a refrigerator at about 10° C. the sperm remains active and retains its full fertilizing power for 24 hours and possibly longer.

The effect of cold storage on the fertilizability of eggs is not known. On several occasions ripe females with intact shells were kept for 3 to 4 days in a refrigerator (about 10° C.) and after that were successfully used in spawning experiments. The effect of cold storage on eggs of the excised ovary has not been studied.

POLARITY OF EGG

The polarity of all molluscan eggs apparently is determined while they are still attached to the wall of the ovarian follicle. Presumably the side on which the food reaches the growing ovocyte becomes the vegetative pole of the mature egg (Raven, 1958).

The metabolic gradient along the egg axis is indicated by a concentration of cytochrome oxidase which Kobayashi (1959) detected with M–Nadi reaction; the activity of the enzyme was observed using Gräff's modification of this method. (The reader not familiar with the reaction and its significance in cytochemical research is referred to the publications of Danielli (1958), Deane, Barrnett, and Seligman (1960), and to a review by Novikoff (1961a, p. 308).) In brief, the localization of oxidative enzymes and their presence in mitochondria can be determined by the staining reactions. The rate of respiration of eggs of *C. virginica* increases with fertilization by a factor of 1.4 (Ballentine, 1940) but in the eggs of the Sydney rock oyster, *C. commercialis*, the rate of respiration increases only at the onset of the first cleavage (Cleland, 1950).

CLEAVAGE

The spermatozoon may enter the oyster egg anywhere. Its path inside the egg cytoplasm toward the nucleus has not been described, and cytological details of the process leading to the fusion of the female and male pronuclei have not been studied. It is probable that the major features of these events are not different from those found in other mollusks. Maturation divisions occur in the oyster egg after the elevation of the fertilization membrane. The germinal vesicle (ovocyte nucleus) breaks down and moves toward the egg's periphery. At temperatures of 22° to 24° C. the first polar body is formed within 25 to 50 minutes after the addition of sperm. The reduction of the number of chromosomes probably takes place during the first meiotic division. This is to a certain extent corroborated by an examination of fertilized eggs of *C. virginica* stained in toto with Feulgen reagent or with acetic orcein. Unfortunately the results are not consistent enough to draw a final conclusion and the question remains unanswered, awaiting a complete cytological study.

The second polar body is formed shortly after the first, within 45 to 70 minutes after fertilization (at 22.5° to 24° C.). The two polar bodies remain attached to the surface of the egg (fig. 319) until the completion of cleavage and emergence of the trochophore.

The first cleavage following the formation of the second polar body divides the egg meridionally into two unequal cells designated as AB and CD (fig. 320). The inequality of the blastomeres is due to the occurrence of a polar lobe. Because the egg appears to consist of three cells this stage received the name trefoil.

The plane of the second division, also meridional, is at a right angle to the first. Both blastomeres divide synchronously and separate into the four quadrants. In fig. 321, drawn from a photograph of a cleaving egg taken from the animal pole, the position of the spindles indicates the plane of new

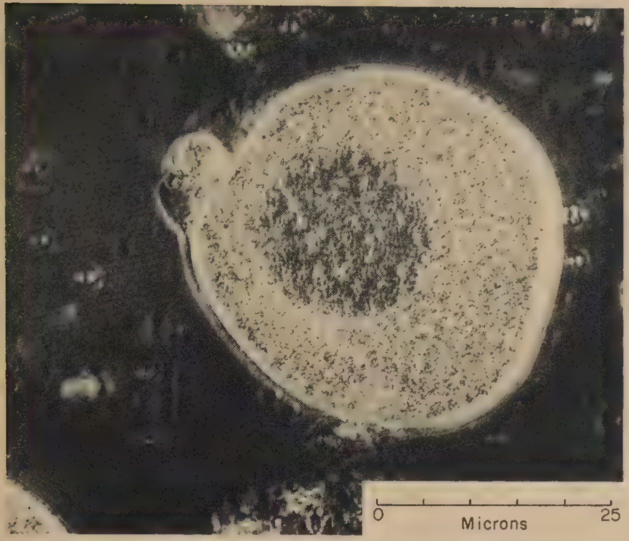

FIGURE 319.—Photomicrograph of a live fertilized egg of *C. virginica* after the formation of two polar bodies (top of egg). High-phase oil immersion lens.

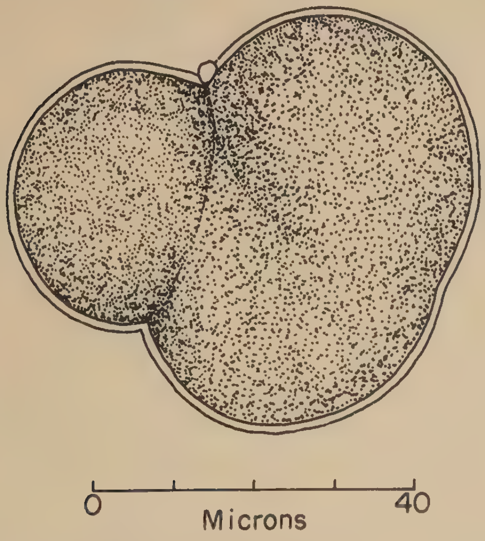

FIGURE 320.—First cleavage division of the egg of *C. virginica* 70 minutes after fertilization. Blastomere AB (left) and CD (right). Polar body on top.

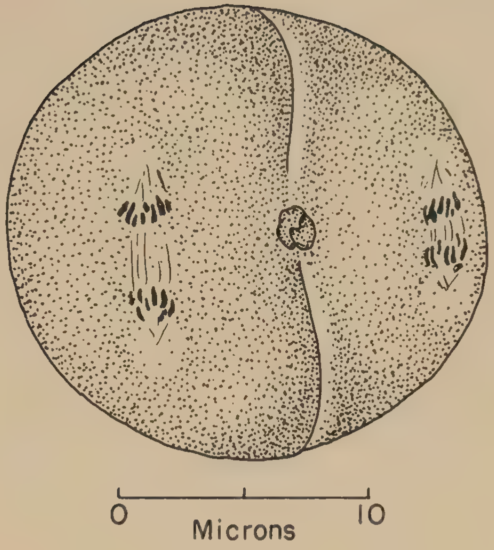

FIGURE 321.—Beginning of second cleavage of the egg. Viewed from the animal pole. Whole mount, Kahle, Feulgen stain.

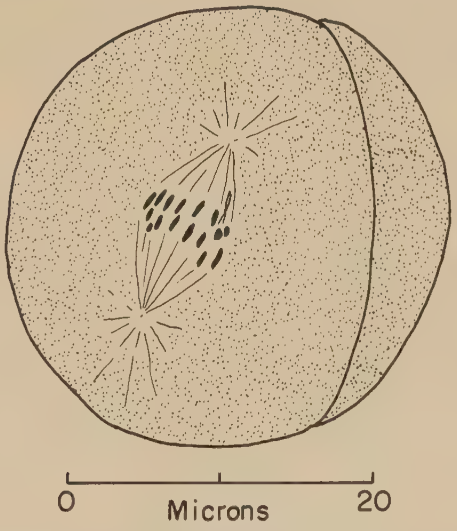

FIGURE 322.—Section of an egg of *C. virginica* at the second cleavage. Beginning of anaphase. Kahle, Heidenhain iron-hematoxylin.

furrows which will intersect the egg into four cells, A, B, C, and D. At this stage the mitotic figures are fairly large and the chromosomes are in a favorable position for examination. In the best sectioned preparations of the cleaving egg, eight daughter chromosomes were counted at the beginning of anaphase (fig. 322). It would be, however, premature to state that the diploid number of chromosomes in *C. virginica* is 8 because on other preparations 7, 9, and 10 were counted.

The third division of each quadrant cuts the cells in the equatorial plane and separates the first quartet of micromeres, small cells at the animal pole, from the macromeres, or larger cells at the vegetal pole (fig. 323).

At the fourth and fifth cleavages, resulting in 16- and 32-cell stages, the micromeres overgrow the macromeres. Only one of the macromeres is visible in the figure 324 and two in 325, which show a side view of an oyster egg at these two stages of development.

Cell lineage, or tracing the developmental history of the cleavage blastomeres through to

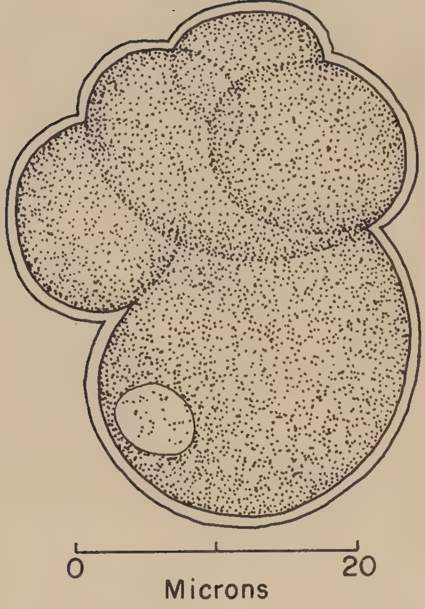

Figure 323.—Third division of fertilized egg of *C. virginica;* side view. Separation of micromeres (on top) from macromeres (bottom). Only one macromere is seen in the plane of view. Live egg.

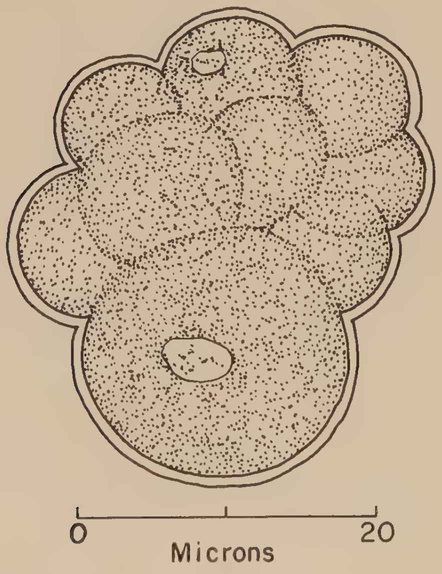

Figure 324.—Fourth cleavage of egg of *C. virginica* and the formation of 8 micromeres (2d quartet). Side view. Drawn from photomicrograph of live cell.

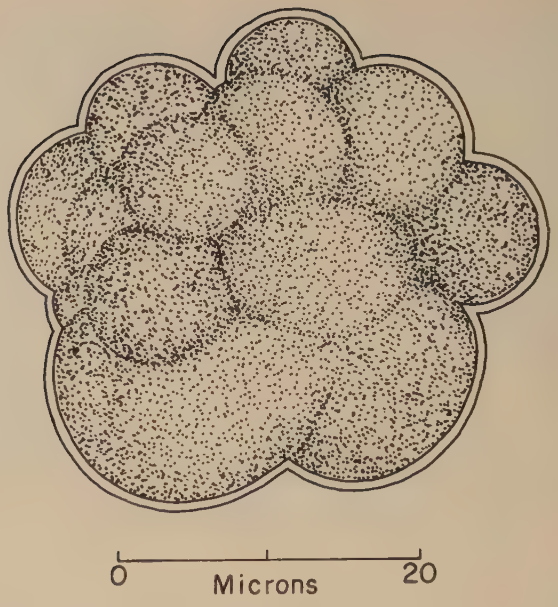

Figure 325.—Fifth cleavage of egg of *C. virginica* and the formation of the third quartet of micromeres (32-cell stage). Side view. Drawn from photomicrograph of live cell.

their ultimate fates as parts of the larva or adult, was first described by Whitman (1878) for the egg of *Clepsine*, and followed by Wilson (1892) for the egg of *Nereis*. The works of Lillie (1895) on the development of Unionidae, Conklin (1897, 1908) on *Crepidula* and *Fulgur*, Meisenheimer (1901a, 1901b) on *Dreissensia* and *Cyclas*, and Wilson (1904a, 1904b) on *Dentalium* and *Patella* constitute major contributions to the embryology of mollusks.

The nomenclature of the cleavage blastomeres, as developed by Wilson (1892), was progressively modified by Conklin (1897), Mead (1897), and Child (1900); the present system is based largely on the work of Robert (1902) on the development of the *Trochus* egg. The system is a combination of letters and numbers by which the blastomeres are identified.

The first four cells or macromeres are designated as A, B, C, and D; in the majority of cases studied D is the largest of the four and is situated at the side which will develop into the posterior portion of the embryo. When the first four blastomeres divide, their daughter cells are denominated 1a and 1A, 1b and 1B, 1c and 1C, and 1d and 1D, the small letters in each case referring to the micromere and the capital letter to the macromere.

In successive divisions 1A divides into 2a and 2A, 1B into 2b and 2B, and so on. When the micromeres divide, 1a is divided into $1a^1$ and $1a^2$, the superscript 1 denoting the daughter cell which is nearest to the animal pole and superscript 2 the one nearer to the vegetal pole. The nomenclature is capable of indefinite expansion, but certain confusion arises when the two daughter cells resulting from the division of one cell lie at an equal distance from the pole. In this case the letters r for right and l for left are used. The practice is, however, not generally followed.

Descriptions of various types of cleavage can be found in volume 1 of MacBride (1914). The equal and unequal cleavages in the spirally cleaving eggs of annelids and molluscs are discussed by Costello (1955) in chapter 2 of Willier, Weiss, and Hamburger (1955).

During division the micromeres of a quartet, viewed from the animal pole, become slightly displaced because the spindles of the dividing cells (not shown in fig. 324 or 325) occupy an oblique position with respect to the egg's axis. At the following divisions the plane of separation of daughter cells is oriented approximately at a right angle to the preceding divisions. The pattern of such cleavage is called spiral. It gives rise to an irregular morula (sterroblastula according to Korschelt and Heider, 1895, from the Greek "sterros" meaning firm) found in annelids (*Nereis*), in some bivalves (Ostreidae, *Teredo*), and in gastropods (*Crepidula*, *Fulgur*, *Nassa*), and others. In all cases the sterroblastula arises from an unequal cleavage during which the micromeres overlie the macromeres, and at each division are slightly displaced to the right (dexiotropic cleavage) or to the left (laeotropic cleavage). Sometimes, as in the case of *Dreissensia*, the second dexiotropic cleavage is followed by a third dexiotropic cleavage after which the normal alternating course is established (Meisenheimer, 1901a). In the case of oyster eggs, as shown by Fujita (1929) for *C. gigas*, the cleavage is laeotropic.

The multicellular stages of a *C. virginica* egg are reached in the course of the sixth and ensuing cleavages (figs. 326 and 327) during which the micromeres divide much more rapidly than the macromeres, become progressively smaller and overgrow the vegetal pole. Approximately at this stage the sterroblastula of an oyster is formed.

Gastrulation begins with epibolic extension of the micromeres. At 22° to 24° C. the stage shown in fig. 328 is reached within 4 to 6 hours.

The cell lineage of *C. virginica* has not been studied; the stages of development of eggs of the species shown in figures 320 to 328 are similar to those previously described by Brooks (1898); Horst (1882) for *O. edulis;* Seno (1929) for *O. denselamellosa;* Hori (1933) for *O. lurida;* Yasugi (1938) for *O. spinosa,* and *C. gigas.* Yasugi

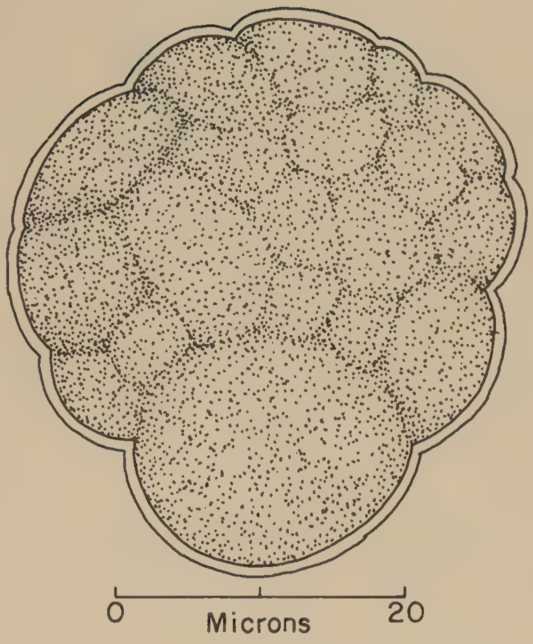

FIGURE 326.—Formation of sterroblastula in *C. virginica* egg; of the four macromeres only two are visible from the side. Micromeres begin to overgrow the vegetal pole. Drawn from photomicrograph of live egg.

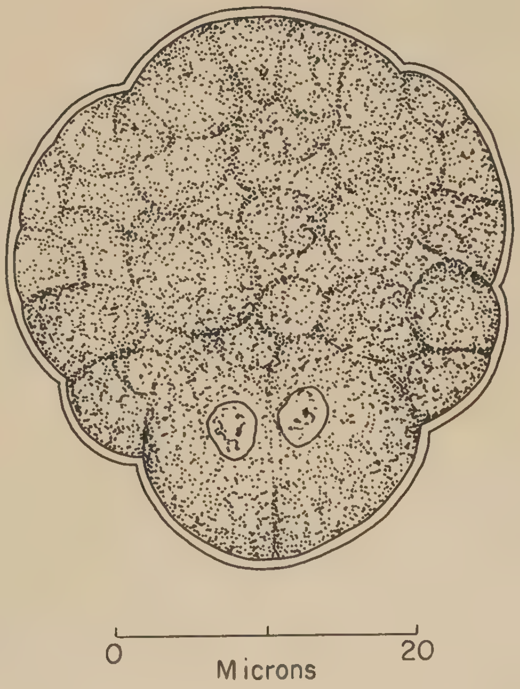

FIGURE 327.—Advanced sterroblastula stage of *C. virginica.* One of the macromeres has divided into two daughter cells of equal size. Drawn from photomicrograph of live egg.

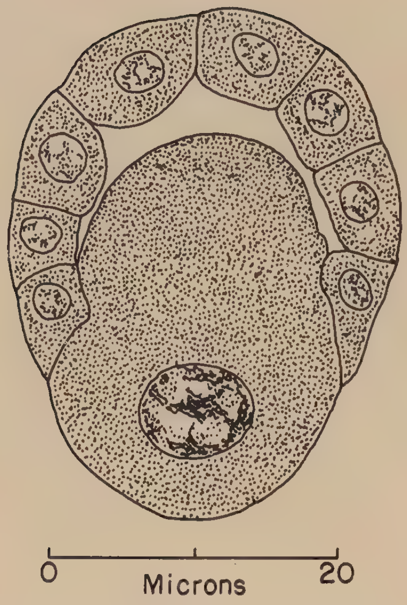

FIGURE 328.—Early stage of gastrulation in the egg of *C. virginica*. Seen at a section of an egg cut along its axis. Heidenhain, iron-hematoxylin.

found that equal cleavage can be induced artificially in eggs of the Japanese oyster by centrifuging for 2 minutes at 1,500 r.p.m. and at the centrifuge radius of 14 cm.

Fujita (1929) gives a brief account of the cell lineage of the eggs of *C. gigas* and states that the mode of cleavage of this species is identical to that of *C. virginica*. The main features described by him are as follows. The first polar body in the fertilized egg of *C. gigas* appears 15 minutes after insemination. At the two-cell stage (fig. 329) the two blastomeres of unequal size, AB and CD, are separated along the meridional plane. Their position corresponds to the anterior (Ant.) and posterior (Pst.) ends of the embryo. The second division, also meridional, separates the four blastomeres A, B, C, and D (fig. 329b).

A and B represent the anterior, and C and D the posterior halves of the embryo, while B and C form its left and A and D its right halves (fig. 329b). The ensuing cleavage starts with the blastomere B and proceeds in laeotropic order to C, D, and A; the resulting daughter cells, the micromeres a_1, b_1, c_1, and d_1, retain the shape of the mother cells but are smaller. The macromere D and micromere d_1 are respectively the largest. The four daughter cells a_1 through d_1 form the first quartet of micromeres located between the macromeres on the dorsal side of the embryo.

The 12-cell stage is initiated by the division of the macromere D; the ensuing larger cell d_2 (fig. 329c) is generally known as the first somatoblast X. (In the system of nomenclature used by American and European embryologists (see p. 346) the 1D cell gives rise to 2d and 2D and the 2d is the X cell.).

The cleavage is continued laeotropically, and the second generations of micromeres a_2, b_2, and c_2 are smaller than the first macromeres. They lie on the outside of the macromeres. The third cleavage of macromeres A, B, and C continues in laeotropic order and results in the micromeres a_3, b_3, and c_3; they are larger than other micromeres. After the third cleavage the macromeres make no further contribution to the formation of micromeres and in the course of development become the entoderm. The first somatoblast (X cell) gives rise to many organs of ectodermal origin. At the 18-cell stage of the embryo the position of cell X and its first divisions mark the beginning of the transition from spiral to bilateral symmetry (fig. 329d).

The mesoderm begins to form at about the 32-cell stage with the appearance of cell 4d, the second somatoblast, also designated as cell M. In bivalves the cell M remains at the surface for a long time, then divides into the two mesodermal teloblasts which sink into blastocoel (Raven, 1958, p. 117). The formation of mesoderm in *C. gigas* has not been followed in detail, but as a rule the mesoderm bands in bivalves remain rather rudimentary (Raven, 1958). Fujita states that the establishment of the three germinal layers in *C. gigas* is completed at the 30-cell stage (fig. 329 e and f).

The gastrula stage is reached in 4 to 6 hours. The cell lineage of *C. gigas* is generally comparable to that described for other bivalves (see: Raven, 1958, p. 70), but for details the reader should consult Fujita's (1929) original text and his drawings.

In about 4 to 6 hours after fertilization, an egg of *C. virginica* reaches the stage (fig. 330) when a few large cilia become visible at the vegetal pole, the oval-shaped body is covered with very

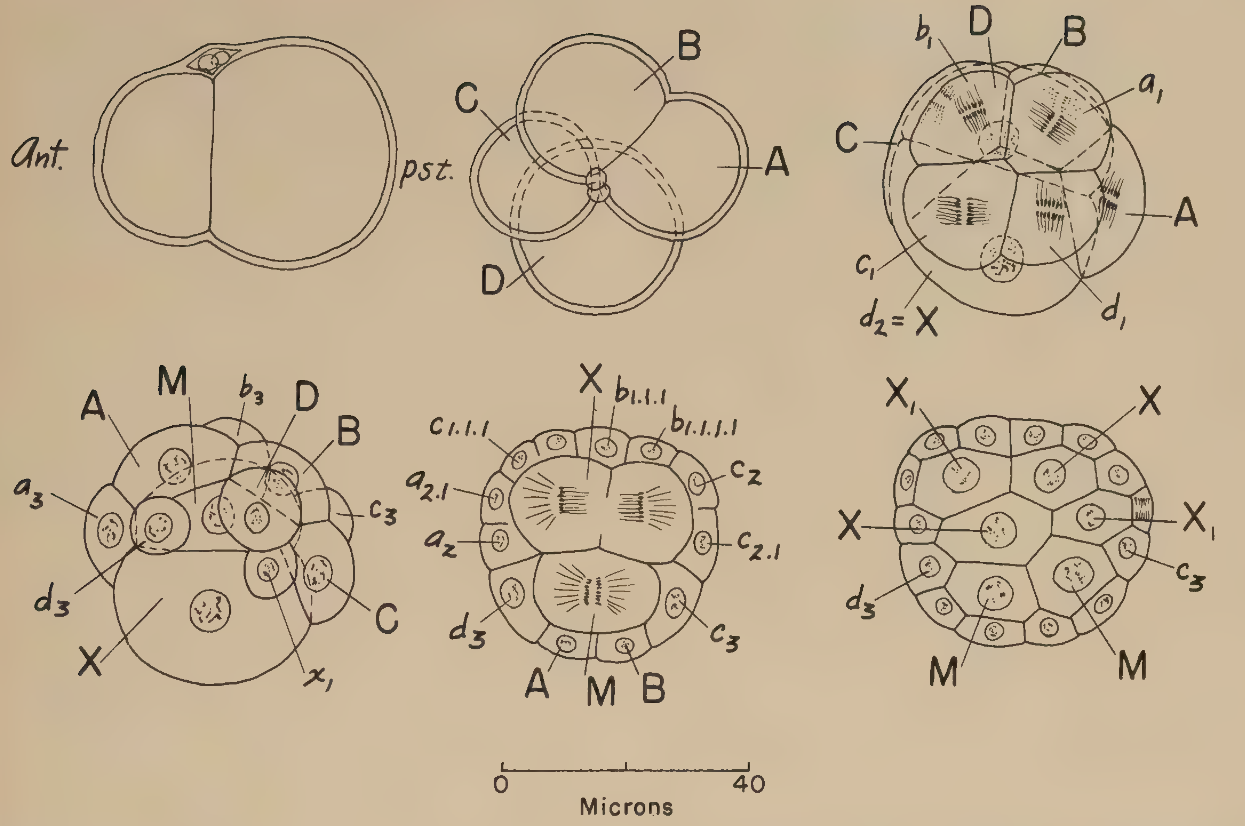

FIGURE 329.—Several stages of development of the egg of *C. gigas.* Redrawn from Fujita, 1929. a—Two-cell stage, Ant.—anterior, Pst.—posterior ends; b—Four-cell stage, formation of blastomeres A, B, C, and D; c—12-cell stage and the formation of the first somatoblast (cell X), viewed from the animal pole; d—embryo viewed from vegetative pole after the formation of the mesomere M; e—cleavage of mesomere M and the first somatoblast X, posterior view optical section; f—advanced stage of development showing the arrangement of the mesomeres M, M, and the somatoblasts, X, X, X_i, X_i, posterior view optical section. Cleavage nomenclature as given by Fujita.

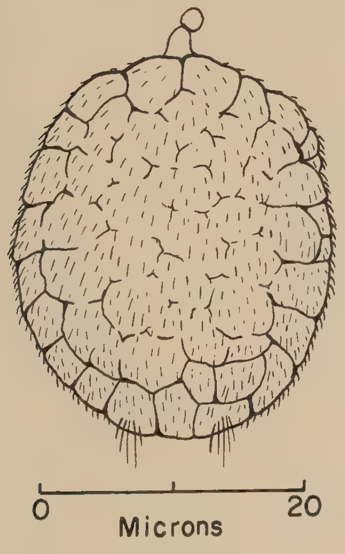

FIGURE 330.—Larva of *C virginica* ready to hatch. Drawn from photomicrograph of live larva.

fine ciliation, and two polar bodies still remain attached to the animal pole. The beating of the cilia is not coordinated at this stage, and the movements of the larva are irregular and spasmodic. A few minutes later a girdle of powerful cilia is formed, the polar bodies are lost, and the larva begins to swim upward (fig. 331). In a finger bowl containing cleaving eggs, the newly hatched larvae appear as white columns rising from the layer of fertilized eggs on the bottom of the container (fig. 332). The larvae can be pipetted off easily, transferred into larger containers and provided with suitable food.

The time required to complete the development of an oyster egg varies, depending on condition of eggs, temperature, salinity, oxygenation of water, and other environmental factors. Records of three sets of observations made in the Woods

Hole laboratory at room temperatures varying from 22.5° to 24.5° C. and salinity of water of 32.2 ‰ are given in table 37. To obtain records of rates of development at different temperatures, several hundred artificially fertilized eggs were placed in each Syracuse dish filled with fresh sea water and covered to prevent evaporation. The debris was removed, and the water contained no unfertilized or cytolyzed eggs.

TABLE 37.—*Observations on the time required for artificially fertilized eggs of* C. virginica *to reach trochophore stage*

All observations were made at Woods Hole in July at room temperatures varying from 23° to 25° C. The time required to reach different stages varied in different groups of eggs. The observations are arranged in two groups: A and B, which differ primarily in the duration of time required to reach rotating blastula and trochophore stages.

	STAGES	
Stage of development	A	B
Fertilization membrane	5 min	10 to 25 min.
First polar body	40 min	25 to 52 min.
Second polar body	1 hr. 10 min	40 to 65 min.
First cleavage	1 hr. 12 min	45 min.
Second cleavage		52 to 120 min.
Third cleavage	2 hr. 10 min	55 to 195 min.
Morula stage		135 min.
Rotating blastula	4 hr	6 hr. 30 min.
Trochophore	5 hr	8 to 9 hr.

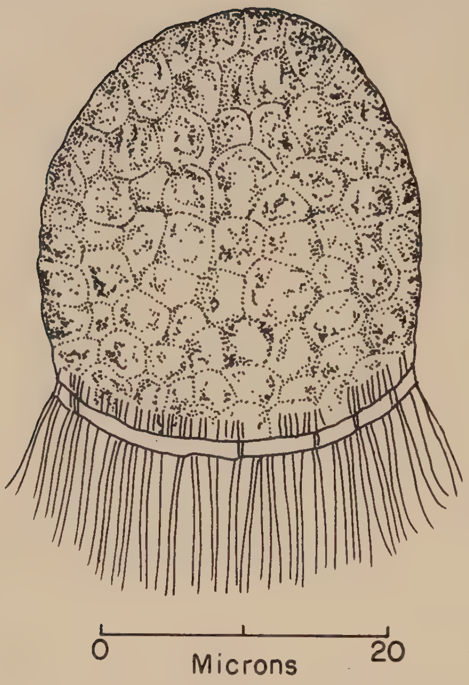

FIGURE 331.—Larva of *C virginica* at the time of its emergence 6 to 6½ hours after fertilization. Drawn from a photomicrograph of a live larva.

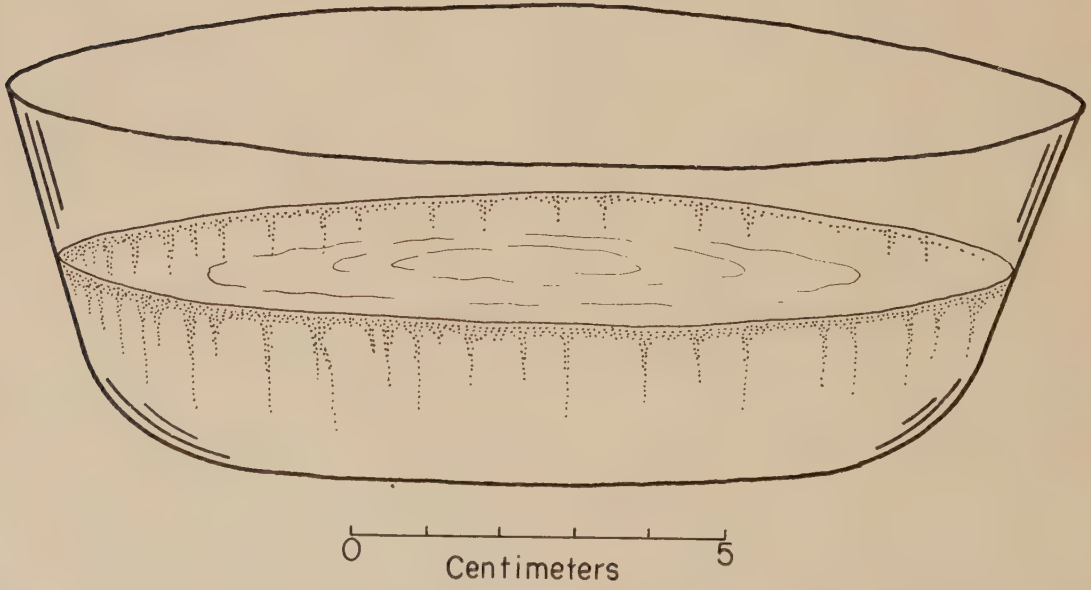

FIGURE 332.—The emergence of larvae of *C. virginica* from fertilized eggs kept in a finger bowl. The free-swimming larvae form columns, which tend to disperse at the surface. Drawn from life.

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CHAPTER XVI

LARVAL DEVELOPMENT AND METAMORPHOSIS

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The anatomical structure of an oyster larva is known primarily from works on the development of *O. edulis* by Horst (1883), Huxley (1883), Dantan (1917), and Erdmann (1935). Fragmentary information regarding other species is found in publications of Stafford (1913) on *O. lurida;* Prytherch (1934) on *C. virginica* and Fujita (1934); on *C. gigas*. Larval histology is described in a comprehensive paper by Erdmann (1935), and fate of larval organs in the metamorphosis of *O. edulis* is discussed by Cole (1938b).

The voluminous literature on the ecology and biology of oyster larvae of *O. edulis* and other species has been reviewed by Korringa (1941) in a lengthy publication which places emphasis on spawning and the setting of oysters. An abundance of ecological data found in the reports of Federal, State, and private organizations concerned with the conservation and management of oyster bottoms, deals mainly with the time of appearance and setting of oyster larvae. Relatively little is known about the factors which control the life and behavior of the larvae, and only a few studies have been made in recent years on larval physiology, nutritive requirements, and metabolism. However, advances in the technique of artificial rearing of oyster larvae from fertilized eggs (Loosanoff and Davis, 1963a,1963b) now make it possible to obtain a continuous supply of larvae of known age regardless of the season of the year. This advantage may stimulate future studies of larval physiology.

ANATOMY OF TROCHOPHORE AND VELIGER

The slightly flattened embryo which forms at the completion of cleavage does not increase in bulk during embryonic development and is about 40μ to 50μ along its dorso-ventral dimension, about the same size as the egg. The two polar bodies may still be attached to some of the embryos and a tuft of robust cilia marks their anterior ends. The larva, which at this moment begins to swim, is called trochophore from the Greek "trochos," a wheel: and "phero," to bear.

The formation of the trochophore results from the epiboly, i.e., the multiplication of small ectodermic cells, their arrangement around the single and much larger macromere, and invagination of the endoderm. At the early stage of larval development, described by Horst (1883) for *O. edulis*, the invagination of endodermic cells (fig. 333, en.) marks the position of the blastopore, bl., (from the Greek "blastos," bud, and "poros," passage). a channel which leads to the archenteron (the cavity of the gastrula). A small invagination of the ectodermic cells at the animal pole of the larva indicates the location of a saddlelike shell gland (sh.g.), which at the later stages gives rise to the larval shell called prodissoconch (from the Greek "pro," before, "dissos," double, and "kongchē," conch or shell).

The invagination of the blastopore (fig. 334, bl.) becomes deeper and narrower; the mesoderm, me., is formed; and the shell gland, sh., increases in size. At the trochophore stage (fig. 335) the blastopore is closed and the mouth, m., is formed above it; the ectodermic cells, ec., develop cilia and are now called the trochoblasts. They form a ciliated ring or prototroch, which functions as an organ of swimming. The position of the prototroch is indicated in fig. 335 by two ectodermic cells, c., with cilia.

As the development of the trochophore advances, the prototroch forms a ciliated crown at

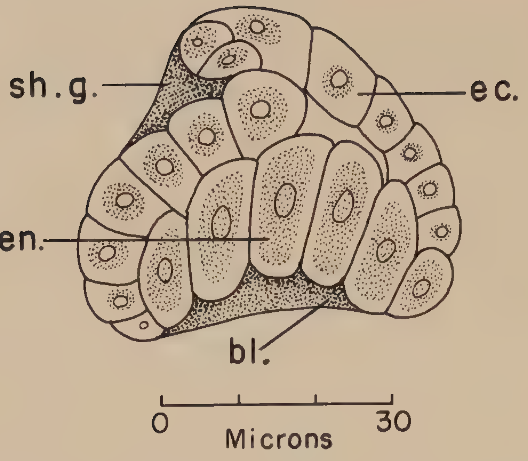

Figure 333.—Optical section of an early stage of development of the larva of *O. edulis* according to Horst. Reproduced from Pelseneer, 1906. bl.—blastopore; en.—endoderm; ec.—ectoderm; sh.g.—rudimentary shell gland.

the ventral side of the larva (fig. 336, pr.). The digestive system consists of the mouth (m.) surrounded by ciliated lobes; large stomach (st.); relatively short intestine (int.); and anus (a.). The thickened central part surrounded by the prototroch is considered to be a rudiment of the cephalic ganglion. The larval shell (sh.) covers a considerable part of the body and is formed into right and left oval valves of equal size and shape joined at the dorsal side of the larva. At the beginning of larval life the beating of the cilia of the prototroch is sporadic and disorganized. Within the next 15 to 20 minutes the larva first rotates around its dorsoventral axis and swims with the ciliated crown directed forward and up toward the surface of the water. The trochophore stage of *C. virginica* is short; in the laboratory at 22° to 24° C. it lasts no longer than 48 hours and in some instances only 24 hours.

The next stage is known as veliger (from the Latin "velum", veil; and "gerere", to carry). A detailed account of the structure and development of bivalve veliger was made by Meisenheimer (1901) for *Dreissensia polymorpha.* MacBride (1914) stated that the development of larvae of *Pecten*, *Teredo*, *Pholas*, *Cardium* and *Ostrea* (including *Crassostrea*) is virtually identical with that of *Dreissensia.* The early larval stages of these forms are so similar that their recognition in plankton samples cannot be made with confidence until their larval shells have been developed. The structure of an early veliger of *O. edulis*, described by Yonge (1926, 1960), is similar to that of *C. virginica* and *C. gigas.* The description given below is based primarily on publications by Yonge (1960) and Erdmann (1935) on *O. edulis.*

The veliger (fig. 337) is a highly complex organism containing several larval organs which disappear with the end of free-swimming life. The most conspicuous among the larval structures is the velum, v., which is formed by an outgrowth of the lateral parts of the prototroch area in two semicircular folds or lobes bearing large cilia along their margins. The prototroch thus develops at

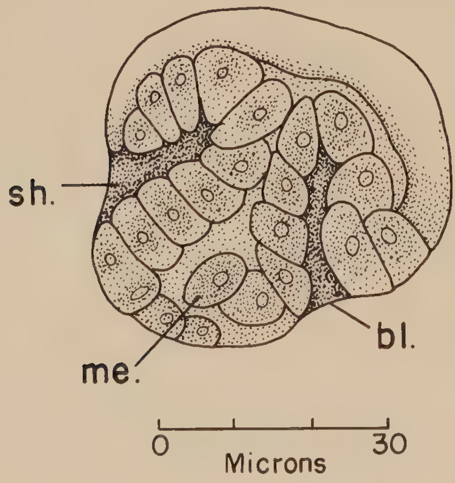

Figure 334.—Optical section of the gastrula stage of development of the larva of *O. edulis* according to Horst. Reproduced from Pelseneer, 1906. bl.—blastopore; me.—mesodermic cells; sh.—rudiment of shell.

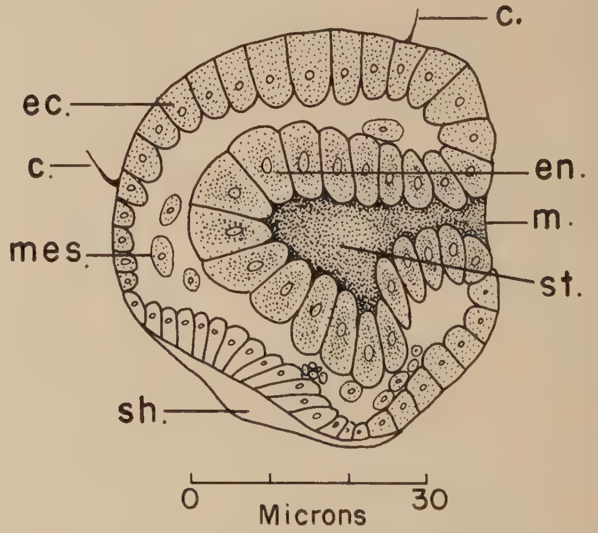

Figure 335.—Optical section of the trochophore larva of *O. edulis* according to Horst, 1883. Reproduced from Pelseneer, 1906: c.—cilia; ec.—ectoderm; en.—endoderm; m.—mouth; mes.—mesodermic cells; sh.—shell; st.—stomach.

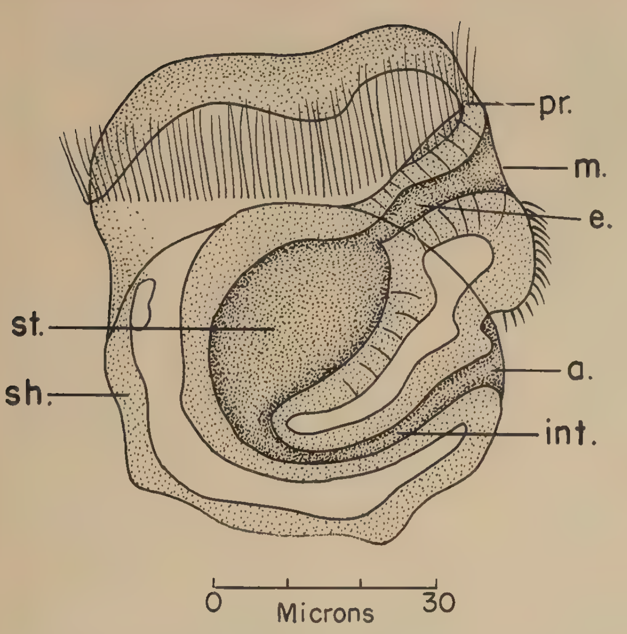

FIGURE 336.—Trochophore of *O. edulis* according to Horst, 1883. Reproduced from Pelseneer, 1906. a.—anus; e.—esophagus; int.—intestine; m.—mouth; st.—stomach; sh.—shell; pr.—prototroch.

the veliger stage into a powerful organ for swimming. During swimming the velum projects between the valves of the shell. It is highly contractile and at the slightest disturbance is withdrawn between the valves by several velar retractor muscles (r.v.), which are attached to the velum and are anchored at the opposite end to the shell.

For examination of the velum, the larvae should be narcotized with menthol, chloral hydrate, or other narcotics, and made transparent with glycerol. The larvae can be satisfactorily narcotized in a small dish by placing tiny crystals of menthol on the surface of the water and allowing them to relax before giving additional crystals. When narcosis appears to be complete, glycerol should be added slowly, drop by drop, to avoid disturbing the larvae and causing them to contract. The method is tedious, time-consuming, and requires a great deal of patience.

Large cilia around the margin of the velum are for swimming; small cilia (not shown in fig. 337), covering the base of the velum carry food particles toward the mouth (m.). A relatively long esophagus (e.) leads to a barrellike stomach (st.), which is in close contact with the glandular structure of digestive diverticula (dig. d.). The crystalline style sac (cr. s.) is at the lower part of the stomach. The intestine (int.) emerging from the stomach makes a single loop and continues into the rectum (r.); the anus (a.) opens into the mantle cavity (m.c.). The foot rudiment (f.) appears as a ciliated outgrowth of the body under the mouth and reaches its full development toward the end of larval life. The anterior adductor muscle (ant. ad.), destined to disappear in older larvae, is conspicuous; the posterior adductor has not yet developed.

The early larvae of *C. virginica* found in plankton samples or developed in the laboratory are oval-shaped and slightly asymmetrical. Because the hinge side of their shells is straight, they are called straight-hinge larvae or D-shaped larvae. Rees (1950) refers to this stage as Prodissoconch I. Dimensions of the larvae vary from 70μ to 75μ in length, i.e., parallel to the hinge side, and from 60μ to 68μ in height, with the greatest distance at a right angle to the hinge side. The prodissoconchs of *C. virginica* are shown in the photomicrographs in fig. 338.

Major changes take place in the appearance and structure of the larva as it grows, reaches its full development, and becomes ready to set. The advanced stages of larval development are called by various descriptive names referring to the most conspicuous morphological change of each stage: umboned larva, eyed larva, adult, and mature larva. The latter expression is frequently used

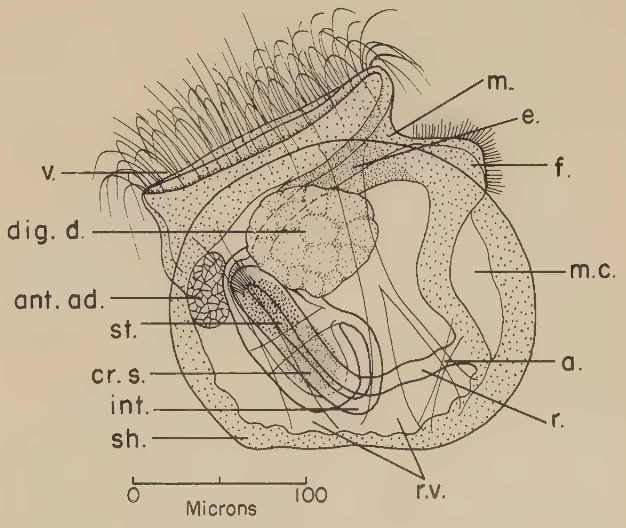

FIGURE 337.—Early free-swimming veliger of *O. edulis*. From Yonge, 1926. a.—anus; ant.ad.—anterior adductor muscle; cr.s.—crystalline style sac; dig.d.—digestive diverticula; e.—esophagus; f.—rudiment of foot; int.—intestine; m.—mouth; m.c.—mantle cavity; r.—rectum; r.v.—velar retractor muscles; sh.—shell; st.—stomach; v.—velum.

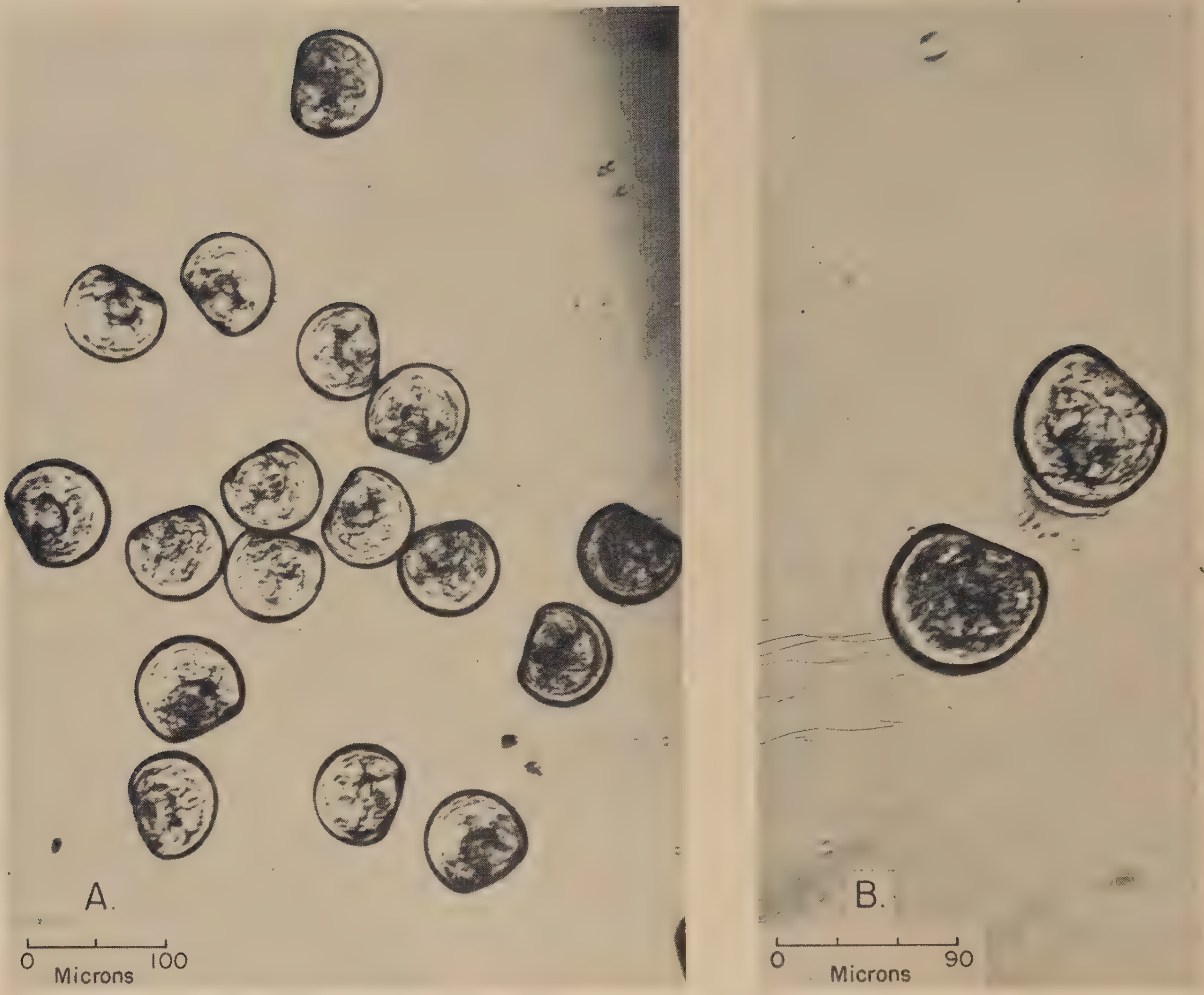

Figure 338.—Photomicrographs of early straight-hinge live larvae of *C. virginica.* A—larvae resting on bottom, shells closed; B—the slightly narcotized larva (upper part) has its velum protruding from the shell; the lower larva has closed its shell and withdrawn its velum.

by English-speaking oyster biologists in spite of the obvious contradiction in applying the adjectives adult and mature to larval stages. The term "velichoncha" proposed by Werner (1940) and adopted by Rees (1950) refers to the advanced stages of development of bivalve larvae, but the expression is not generally used in malacological literature. The name "pediveliger" was proposed by Carriker (1961) to designate the "swimming-creeping" stage of clam larva, *Mercenaria* (*Venus*) *mercenaria.* The term deserves to be accepted in malacological literature because it indicates the major character, i.e., the presence of a foot, and is applicable to many bivalve species, including oysters, in which a larval foot appears during the planktonic period.

As the larva grows its valves become deeper and almost circular. The hinge develops two bulgings or umbones, the one on the left side larger than its opposite number. At these stages the umbones bend toward the posterior end of the shell, which at this time has pronounced concentric rings, is heavy, and obscures the organs under it. In swimming the umbo larva protrudes its large ciliated foot forward. The larvae of *C. virginica* now have diameters of more than 300 μ in both length and height. The photomicrograph in figure 339 shows the side view of the advanced

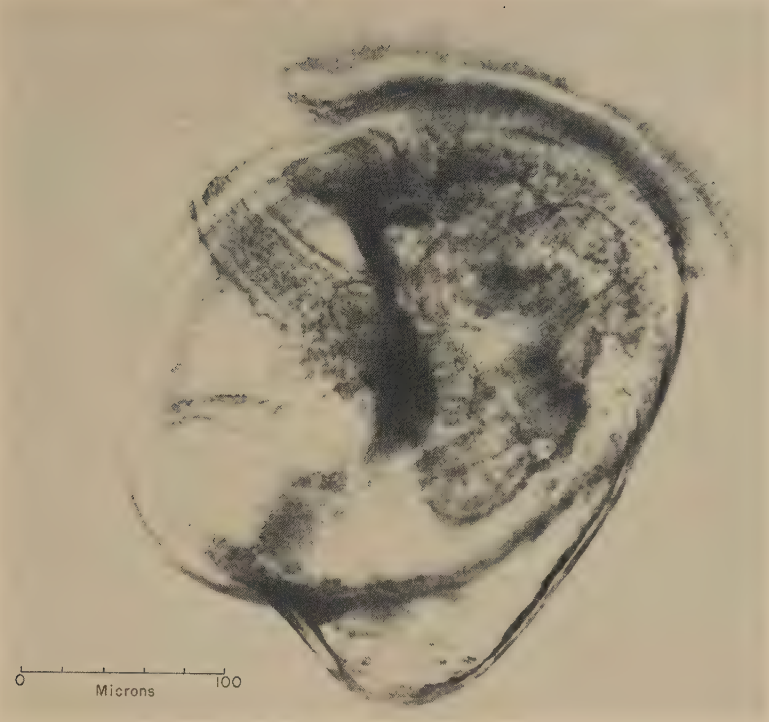

FIGURE 339.—Photomicrograph of live, slightly narcotized umbo larva of *C. virginica*.

umbo larva of this species, slightly narcotized to reduce its movements.

The anatomy of fully developed oyster larva is known primarily from the work of Erdmann (1935) on *O. edulis*. Figure 340, reproduced from his publication, shows the velum (v.) with a crown of powerful cilia arranged in a preoral ciliated circle, and a ciliated aboral belt or zone covered with small cilia (ab.c.).

Four pairs of velar retractors (r.v.) withdraw the velum. The muscle bands consist of bundles of cross-striated fibers along the dorsal side of the body. The cross striation of the velar retractors of oyster larva is typical for rapidly contracting muscles. In swimming the veliger rapidly changes the degree of expansion and the position of the velum, and withdraws the organ with great rapidity when the valves begin to close. The striated muscles in the larva indicate the high degree of specialization of larval organs needed for the organism to function effectively. The muscles of an adult oyster are nonstriated. Their contractions are relatively slow and do not require the mechanism typical for the rapid movements of the free-swimming organism. The apical sense organ (a.p.o.) ("Scheitelorgan", according to Erdmann) and cerebral ganglion occupy a central position in the crown of the velum. The function of the apical organ is not known.

A new feature in the larval anatomy, not present at earlier stages, is a well-developed foot (f.) covered with strong cilia. The foot is highly contractile and can be withdrawn by its retractor muscle (f.r.). A byssus gland (b.g.) with a small duct opening into the mantle cavity (m.c.) is located at the base of the foot. Both the foot and the gland are typical larval structures which disappear after performing their function during the attachment. Two muscles, the anterior and posterior adductors (ant.ad. and post.ad.), close the valves. The mouth (m.) is surrounded by a ciliated ridge which develops into the labial palps. The esophagus (e.) leads to the stomach (st.), part of which is covered with the gastric shield (g.sh.). The crystalline style sac (cr.s.) and digestive tubules have greatly increased in size, and the ciliary motion inside them is accelerated. The intestinal tract (int.) forms a loop and ends in the rectum (r.), which has an anal (a.) opening into the mantle cavity (m.c.). The rudiment of heart and kidney is represented by a group of cells (h.r.) shown in figure 340 above the

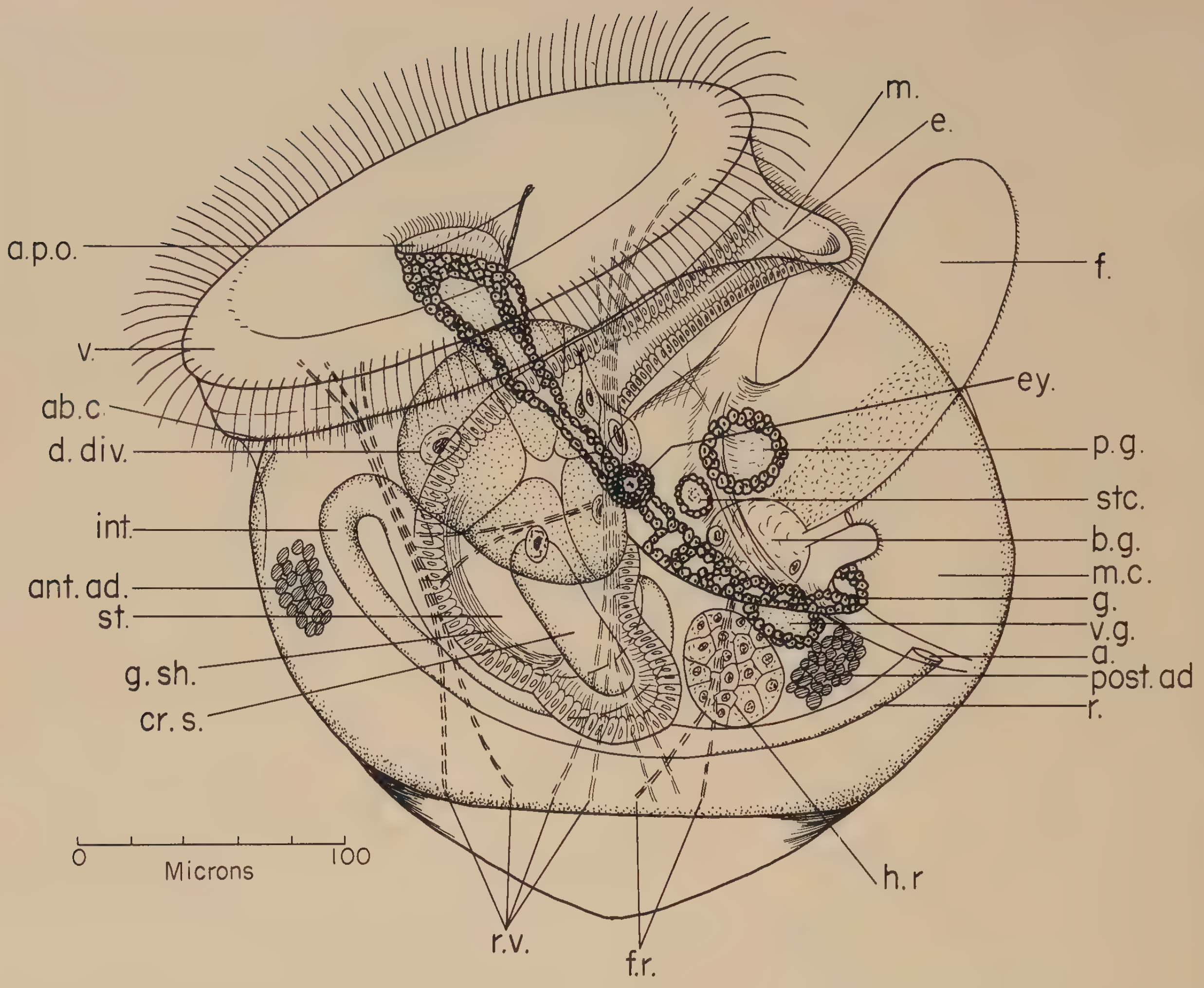

FIGURE 340.—Fully developed larva of *O. edulis* viewed from the right side with velum and foot at the ventral side in the uppermost position, typical for swimming. According to Erdmann, 1935. a.—anus; ab.c.—aboral belt of cilia; ant.ad.—anterior adductor muscle; a.p.o.—apical sense organ and ganglion; b.g.—byssus gland; cr.s.—crystalline style sac; d.div.—digestive diverticula; e.—esophagus; ey.—eye; f.—foot; f.r.—foot retractor muscles; g.—gill rudiment; g.sh.—gastric shield; h.r.—heart and kidney rudiment; int.—intestine; m.—mouth; m.c.—mantle cavity; p.g.—pedal ganglion; post.ad.—posterior adductor muscle; r.—rectum; r.v.—velar retractor muscles; st.—stomach; stc.—statocyst; v.—velum; v.g.—visceral ganglion.

rectum. The gill rudiment (g.), located between the base of the foot and heart rudiment, consists of a series of short, tubular channels. The pedal ganglia (p.g.), a round structure at the base of the foot, disappear with the dissolution of the foot. The visceral ganglion (v.g.) appears in its permanent position at the ventral side of the posterior adductor. The larval sense organs comprise a pair of statocysts (stc.) in the foot tissue and a pair of dark pigmented eyes (ey.) which develop toward the end of larval life. Their presence in the free-swimming larvae indicates the approaching of setting and metamorphosis.

The nervous system of the larva, shown diagrammatically in figure 341, is more complex than that of the adult oyster. It contains the pedal ganglia (ped.g.), which are absent in the adult; the pleural ganglia are present as a separate structure and are connected to the statocysts (syc.) and eyes, which disappear without a trace during metamorphosis. The visceral ganglia (visc.g.) of the larvae are less conspicuous than in the adult. All these organs are obviously necessary to a free-swimming organism, and some of them disappear with the loss of locomotion and the change to a sedentary mode of living.

The anatomy of the fully developed larva of *C. virginica* is similar to that of *O. edulis*. Figure

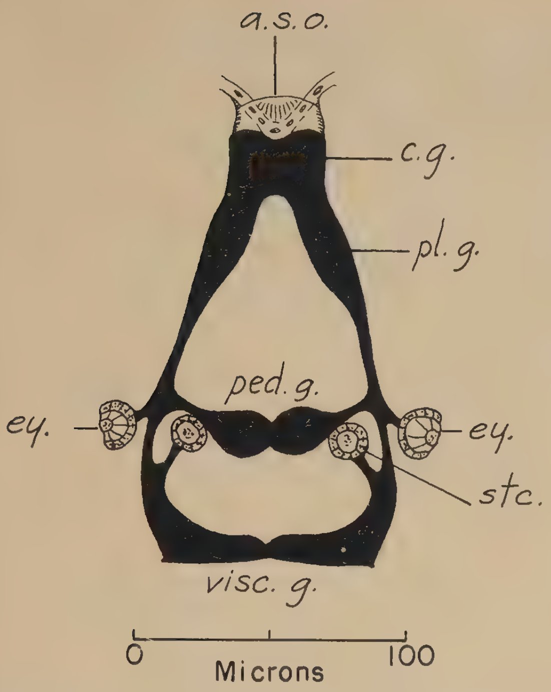

FIGURE 341.—Diagram of the nervous system and sense organs of fully developed larva of *O. edulis*. According to Erdmann, 1935. a.s.o.—apical sense organ; c.g.—cerebral ganglion; ey.—eye; ped.g.—pedal ganglia; pl.g.—pleural ganglion; stc.—statocyst; visc.g.—visceral ganglia.

342 shows the structure of the larva as it appears in the narcotized live specimen. The drawing is a composite from a number of photographs of live larvae taken with the microscope magnification of about 100 X, and from examination under higher power of specimens mounted in glycerin jelly. Only the organs visible under these conditions are shown in this illustration. The larvae were at the last stage of development, over 300μ in height, with eyes (ey.) and a well-developed foot (f.). The velum was large with long cilia at the top and a row of shorter ones forming an aboral circle (ab.c.) at the base. The apical organ could not be seen in the whole mount preparations. The retractors of the velum (r.v.) were well developed. As in *O. edulis* they consisted of rapidly contracting bands of striated fibers. When the velum is completely withdrawn within the shell cavity, the valves close and the larva drops to the bottom. In a contracted state the different organs become undistinguishable. The well-developed foot (f.) contains a large byssus gland (b.g.). During swimming it protrudes between the valves and is kept in the direction of swimming. The tip of the foot frequently turns right or left and up and down while the larva is swimming. This behavior suggests that it serves to orient the movements. At the last phase of larval life the foot is used for crawling over the hard surface where the young oyster will finally attach itself. The funnel-shaped mouth (m.) leads to a narrow and long esophagus (e.), which opens into a barrellike stomach (st.) partially surrounded with massive and dark digestive diverticula (d.div.). The intestinal loop (int.) and rectum (r.) are similar to those described for *O. edulis*. Both adductor muscles (ant.ad. and post.ad.) are well developed. The gill rudiment (g.) appears as a strand of cells in the mantle cavity, and the beating of the heart (h.), located between the stomach and the posterior adductor, can be seen in live specimens. At 24° C. the beating of the heart is rapid, varying from 80 to 100 pulsations per minute.

Food apparently is gathered by the ciliary mechanism of the velum, and small food particles can be observed entering the esophagus and moving inside the stomach where they are rotated by the ciliary epithelium. The ciliated apparatus of the gills has not yet fully developed, and food is gathered only by the aboral circle of the velum (ab.c.) and by the labial palps around the mouth. The statocysts (stc.) and the eye (ey.) are well formed. In a tangential section the eye of *C. virginica* appears as a transparent lens surrounded by a circle of darkly pigmented cells (fig. 343). The dark band is a short branch of a nerve leading to the eye.

The highly developed ciliary mechanism of the velum and the rapidly contracting velar retractors are essential to the life of a free-swimming larva. Their structure appears to be better developed than those of the muscles and ciliary epithelium of adult oysters. Electron microscopy reveals that the ciliated cells of the velum have a highly complex system of basal bodies and rootlets with distinct periodicity (fig. 344). Intercommunication between adjacent cilia through the basal bodies and their branches provides a system for the coordination of ciliary motion. The complexity of the ultrastructure conforms to the complexity of the ciliary activity of the velum, making it possible for the larva to swim in any direction, to turn around, or instantaneously to stop ciliary activity. The ciliated cells of the velum are very large; their surface is covered with microvilli, and

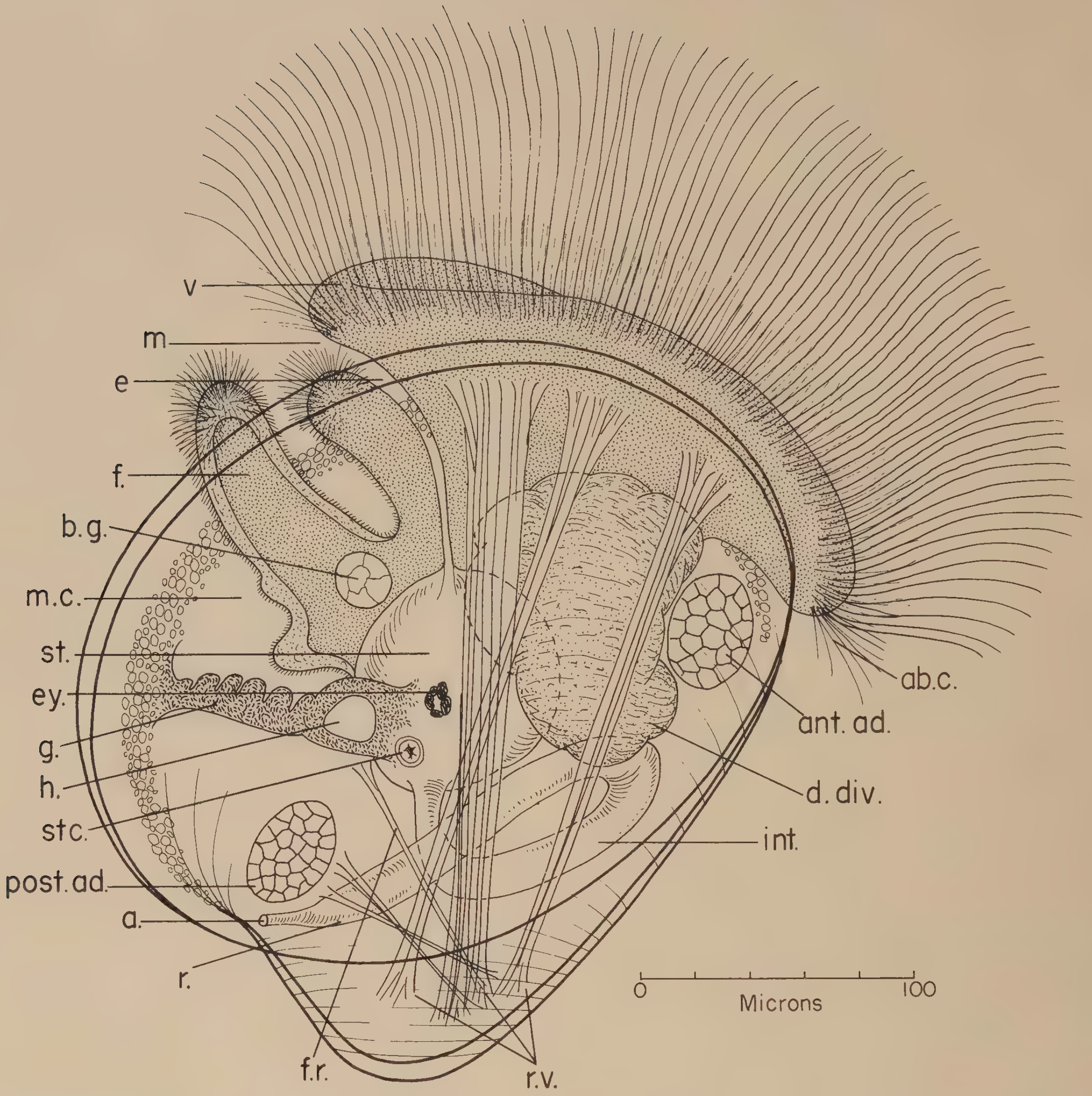

FIGURE 342.—Optical section of fully developed larva (pediveliger) of *C. virginica* viewed from the left side, in swimming position. Composite drawing from a number of photomicrographs of live, slightly narcotized larvae and whole mounts in glycerol. a.—anus; ab.c.—aboral circle of cilia; ant.ad.—anterior adductor muscle; b.g.—byssus gland; d.div.—digestive diverticula; e.—esophagus; ey.—eye; f.—foot; f.r.—foot retractor muscles; g.—gill rudiment; h.—heart; int.—intestine; m.—mouth; m.c.—mantle cavity; post.ad.—posterior adductor muscle; r.—rectum; r.v. velar retractor muscles; st.—stomach; stc.—statocysts; v.—velum.

they contain large oval mitochondria close to the rootlets.

The high degree of specialization of larval organs may be regarded as an adaptive organization of a free-swimming organism to its environment and may have no phylogenetic significance. The pelagic larva of a bivalve has a double task: to distribute the species and grow into an adult. The performance of these tasks requires the maintenance of an equilibrium between the locomotive efficiency and the weight to be carried; this maintenance is accomplished by the development of the velum. As the shell grows and becomes thicker and heavier, the task of swimming becomes more difficult, and the fully grown larva sinks to the bottom more rapidly and possibly more often

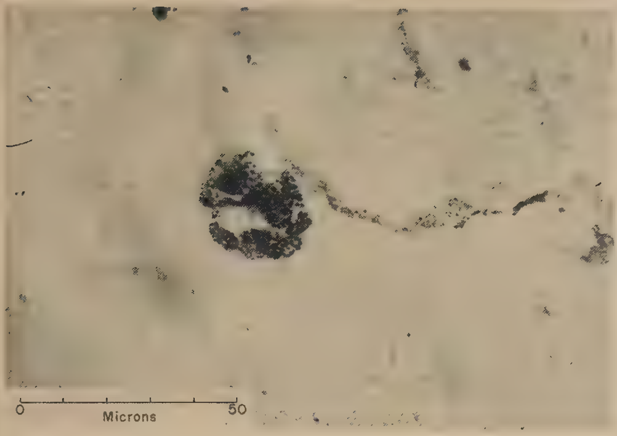

FIGURE 343.—Photomicrograph of a tangential, slightly slanted section of the larval eye of *C. virginica* preserved in osmic acid.

than it does at the straight-hinge stage. When the larva attaches to the substratum, the velum and the foot are no longer needed. Their disappearance marks the transition from free-swimming to a sedentary mode of life. Garstang (1929) expresses the correct opinion that larval organs should be regarded as an adaptation to the condition of life during development and need not affect the organization of the adult. His charming book on larval forms (Garstang, 1951) summarizes in a somewhat unorthodox way the ideas and theories concerning the significance of various larval forms in the evolution of aquatic animals.

MORPHOLOGY OF LARVAL SHELL

The morphology of the larval shell differs from that of an adult oyster primarily in the greater

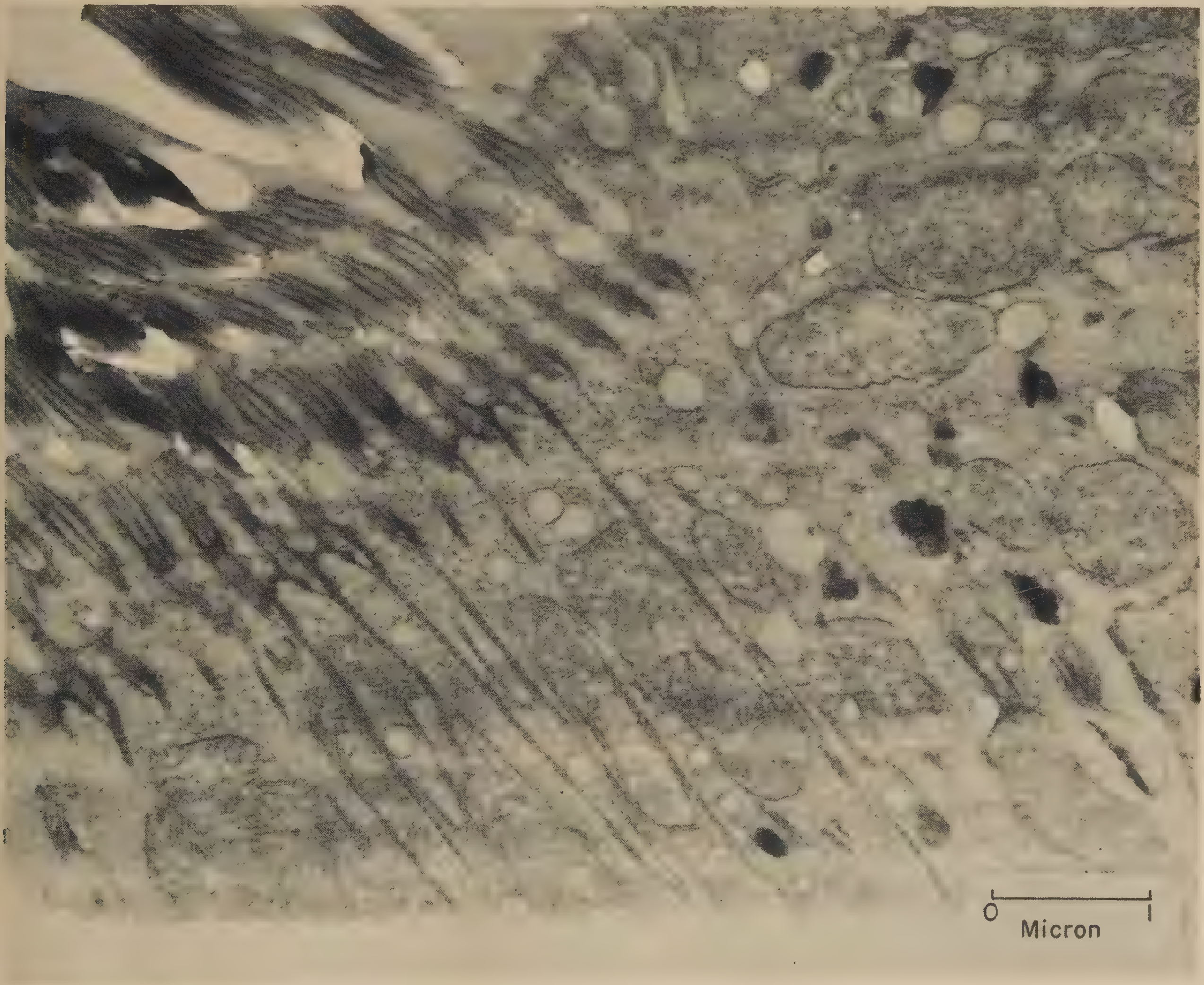

FIGURE 344.—Electron micrograph of a tangential section of a portion of a ciliated cell of the velum of the larva of *C. virginica.*

complexity of the hinge apparatus of the prodissoconch. The hinge ensures the exact closure of the valves and prevents them from sliding on each other under uneven pressure. Consequently, as the larva grows the hinge apparatus increases in strength and complexity. According to Bernard (1898), who made an extensive study of the ontogeny and morphology of larval shells of bivalves, the straight part of the dorsal shell margin thickens to form a provinculum (from the Latin "pro", before, and "vinculum", bond or band) or primitive hinge. The provinculum (by definition) always bears teeth or is shaped into toothlike projections which fit into the corresponding gaps of the opposite valve.

On the basis of the hinge structure Rees (1950) proposed a system of classification of bivalve larvae that greatly facilitates their recognition in plankton samples (fig. 345). He postulated that each superfamily of bivalves has a distinct type of larval hinge; that the shape of the hinge is typical as a generic and species characteristic; and that the texture of the larval shell can be used in certain cases in the recognition of a species. In the families Pteriacea and Ostreacea the hinge apparatus consists of a series of small, uniform teeth (taxodont teeth) in the central portion of the strip and a few larger rectangular teeth with clear gaps between them (fig. 346) at the posterior section. The distinguishing features of the species of these families is the absence of lateral and special teeth and of flanges, i.e., the thick edges of the valves on both sides of the provinculum. The ligament lies between the posterior rectangular teeth and the taxodont strip (Bernard, 1898; Borisiak, 1909). In *O. edulis* there are some large corrugations anterior to the taxodont teeth (Rees, 1950), but their taxonomic value is doubtful.

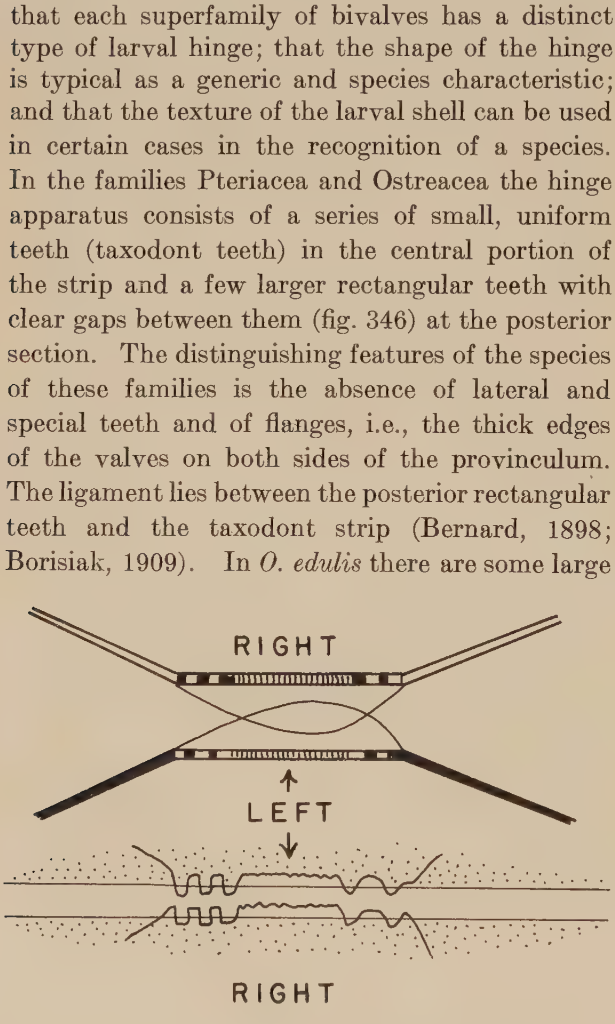

FIGURE 345.—Type of hinge of the families Ostreacea and Pteriacea. Redrawn from Rees, 1950.

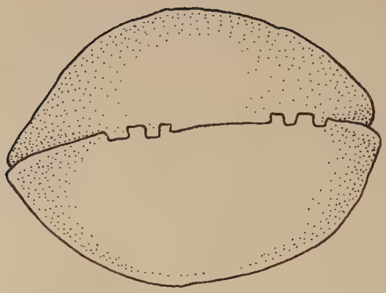

FIGURE 346.—Drawing of a 5- to 6-day-old prodissoconch of *C. virginica*, 70 μ long, examined from the dorsal side.

Differing arrangements and numbers of taxodont teeth in the shells of various species of oyster larvae are used for their identification. The straight-hinge line of a 5- to 6-day-old larva of *C. virginica* grown in laboratory culture has two groups of rectangular teeth that can be clearly seen by examining the shell from the dorsal side (fig. 346). At this stage there is only a slight difference between the upper (right) and lower (left) valves. The difference becomes more pronounced as the larva reaches the umbo stage.

In a series of papers Ranson (1943, pp. 52–58) attempted to establish the classification of all adult Ostreacea on the basis of the fully developed prodissoconchs. Essentially this work was based on the investigations published long ago by Bernard (1898) and Borisiak (1909). Ranson (1960) separates the oysters into three genera: *Pycnodonte*, *Crassostrea*, and *Ostrea*. Each genus, according to his data, is determined by the character of the final prodissoconch hinge and the position of the ligament in relation to the hinge. He concludes his paper with the statement that "as far as the Ostreidae are concerned, the species can now be established on a firm basis, which so far had never been done by studying the adult." The list published by Ranson includes 5 species of *Pycnodonte*, 12 species of *Crassostrea*, and 19 species of *Ostrea*. Unfortunately the diagnosis is given only for each genus without descriptions of taxonomic characters which are shown by the illustrations. The drawings referring to the five species found in the waters of the United States are reproduced in figures 347 through 351. Ranson's text does not include the larvae of *C. gigas* or *O. equestris*.

Ranson states also that the oysters can be correctly identified by the structure of the larval

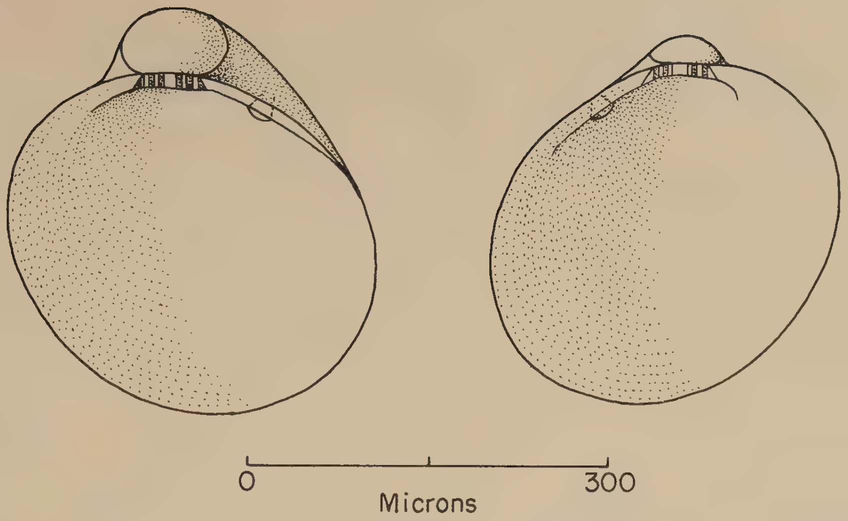

FIGURE 347.—Prodissoconch of *C. virginica* (Gmelin). Inner view of the valves. Left valve on the left and right valve on the right. Knoblike structure indicates the location of the ligament. From Ranson, 1960.

shells still visible on the shells of adults. Examination of the many shells of adult *C. virginica*, *C. gigas*, *C. rhizophorae*, and *O. equestris* in my collection did not reveal the structure of their larval shells, which in many instances appeared to be eroded or were missing. It is doubtful that Ranson's method of identification of adults by their larval shells will gain acceptance by taxonomists. Comparison of his illustrations of the closely related species, such as *C. virginica* and *C. rhizophorae*, indicates no significant differences between the two. On the other hand, his set of drawings of pelagic prodissoconchs may be useful for planktonologists, at least for separating the three genera of oyster larvae.

ATTACHMENT AND METAMORPHOSIS

Larval life ends when the oyster attaches itself to a substratum. This event is called setting, settlement, or spatfall; the different expressions are used interchangeably and are synonymous. The word setting is commonly used by American biologists and oyster growers; the expressions settlement and spatfall are more frequently found in Canadian and British publications. The term setting will be used throughout this text except in quotations from other authors.

The fully developed larva of *C. virginica* swims with its foot projecting between the valves. When the foot touches a solid surface, the larva

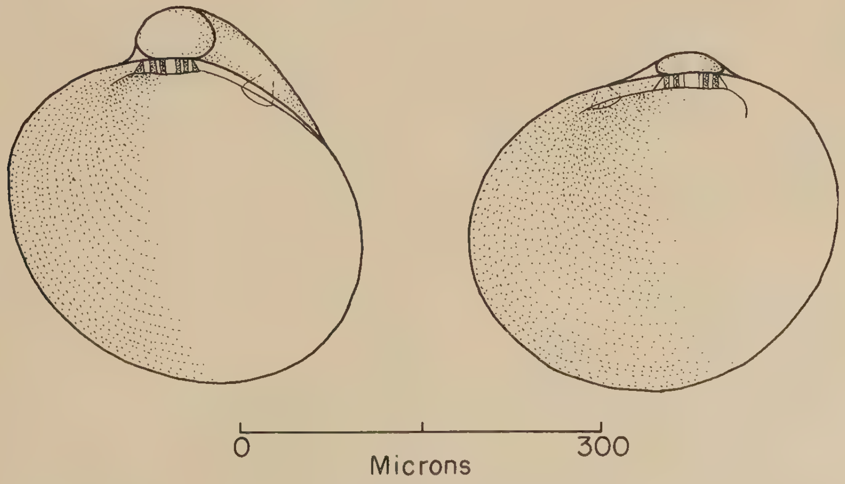

FIGURE 348.—Prodissoconch of *C. rhizophorae* (Guilding). Arrangement as in figure 347. From Ranson, 1960.

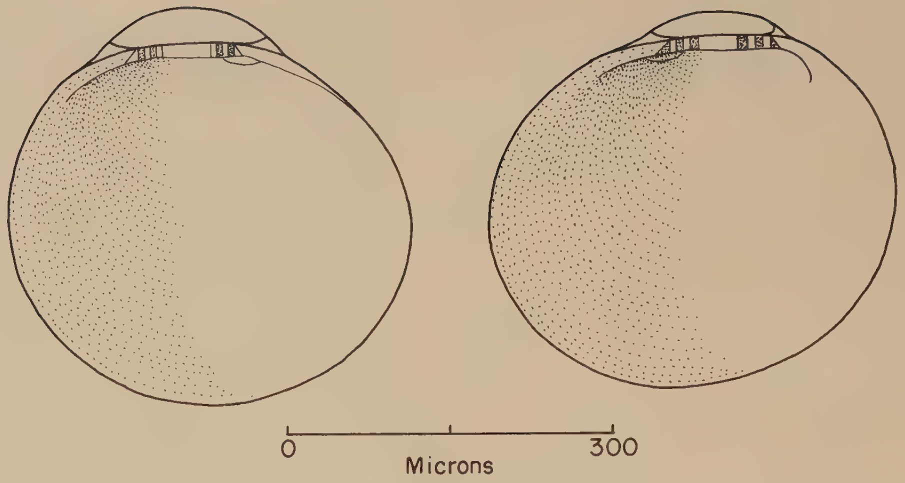

FIGURE 349.—Prodissoconch of *O. edulis* Linné. Arrangement as in figure 347. From Ranson, 1960.

stops swimming, the velum is partially withdrawn, and the larva begins to crawl on its foot. This behavior may be changed suddenly by the resumption of swimming; the foot may be withdrawn, the velum expands again, and the larva swims away. When it is ready to set, the larva crawls until it encounters suitable condition for final attachment.

Phases of setting of *C. virginica* were recorded by a motion picture camera nearly 30 years ago (Prytherch, 1934) and the photographs were recently reproduced by Medcof (1961, p. 19). To facilitate photography, the larvae were cemented with marine glue on their left valves to a glass slide which was tilted at a 45° angle. Under such conditions the larvae had no free choice in selecting the place for attachment, and the records obtained in this manner do not represent normal behavior. The attachment of fully developed larvae can be observed, however, by placing them in sea water in a petri dish and observing their behavior with a binocular microscope.

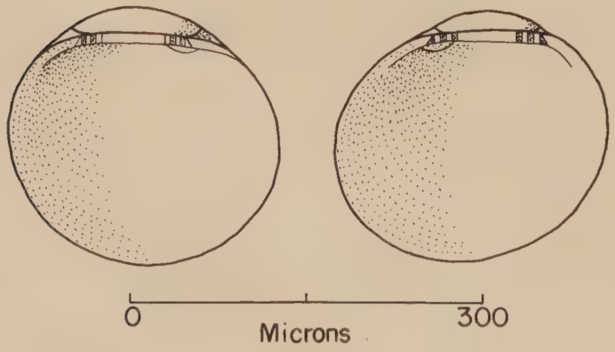

FIGURE 350.—Prodissoconch of *O. lurida* Carpenter. Arrangement as in figure 347. From Ranson, 1960.

The foot of the larva extends forward, its tip attaches temporarily to the substratum, and the whole body is pulled over by the contraction of the foot. The direction of crawling changes and occasionally reverses as the foot extends at different angles. The movement continues for some time, gradually becoming shorter and slower. Finally the foot extends far beyond the edges of the shell, the larva turns sideways with its left valve touching the substratum, and comes to a standstill. The attachment is made permanent when the byssus gland discharges a cementing fluid, which sets within a few minutes (Nelson, 1924). A similar process takes place in the setting of *O. edulis* and is probably common to other species of oysters.

The change from larva to juvenile oyster (spat) then begins immediately. The process of this metamorphosis is better known for *O. edulis* than for other species of oysters, for it has been studied by Davaine (1853), Huxley (1883), and more recently by Cole (1938b). The work of early European zoologists influenced the study of the American oyster to such an extent that in several instances the description of the metamorphosis of *C. virginica* has been repeated almost verbatim from studies on *O. edulis* with only slight changes (Ryder, 1883; Jackson, 1888, 1890). A somewhat more detailed account of the transformation of larva into spat of *C. virginica* and *O. lurida* was given by Stafford (1913).

During the metamorphosis the larval organs

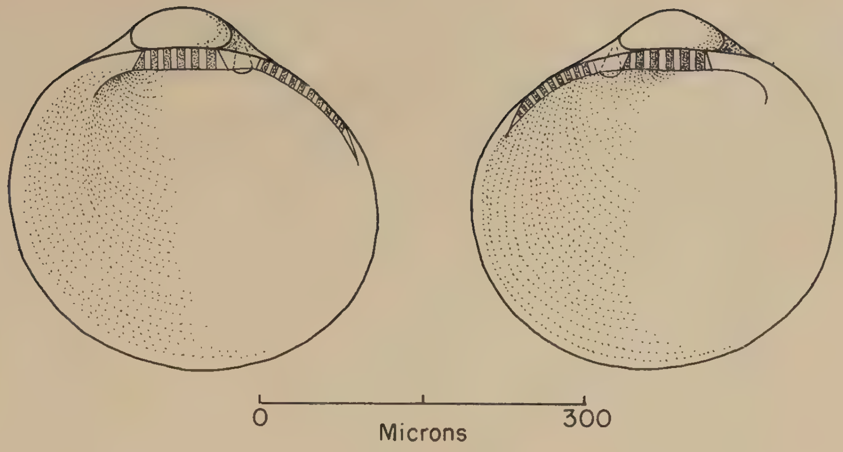

FIGURE 351.—Prodissoconch of *Pycnodonte hyotis* (L.). Arrangement as in figure 347. From Ranson, 1960.

disappear and there is an anatomical reorganization of the permanent organs. At this time the relative size of the organs and their orientation are changed. The extent of topographical changes in the relative position of organs during the transition from larva to spat can be appraised by comparing the position of some of the larval organs with that in the adult oyster. In figure 352 the principal organs of the early larval (1), fully grown larva (2), and of the juvenile oyster or spat (3) of *O. edulis* are shown diagrammatically in three drawings oriented along the dorso-ventral axis. The mouth (m.), nearly ventral in the larvae (1, 2), has shifted counterclockwise (when viewed from the right side of the oyster) about half the periphery of the larva and in the spat occupies an area in the anterio-dorsal part near the hinge. The position of the anus (a.) changes in the same direction, from the dorso-posterior part in the larva to dorso-ventral in the adult. The retractor muscles of the velum (r. v.) disappear by the end of the larval period and in the spat and adult are replaced by the radiating and marginal pallial muscles.

The most conspicuous and rapid changes take place in the velum. Davaine (1853) suggested that in *O. edulis* the velum is cast off about the end of the larval period, a conclusion not confirmed by Ryder (1883) and Stafford (1913). Illustrations by Meisenheimer (1901) of the larva

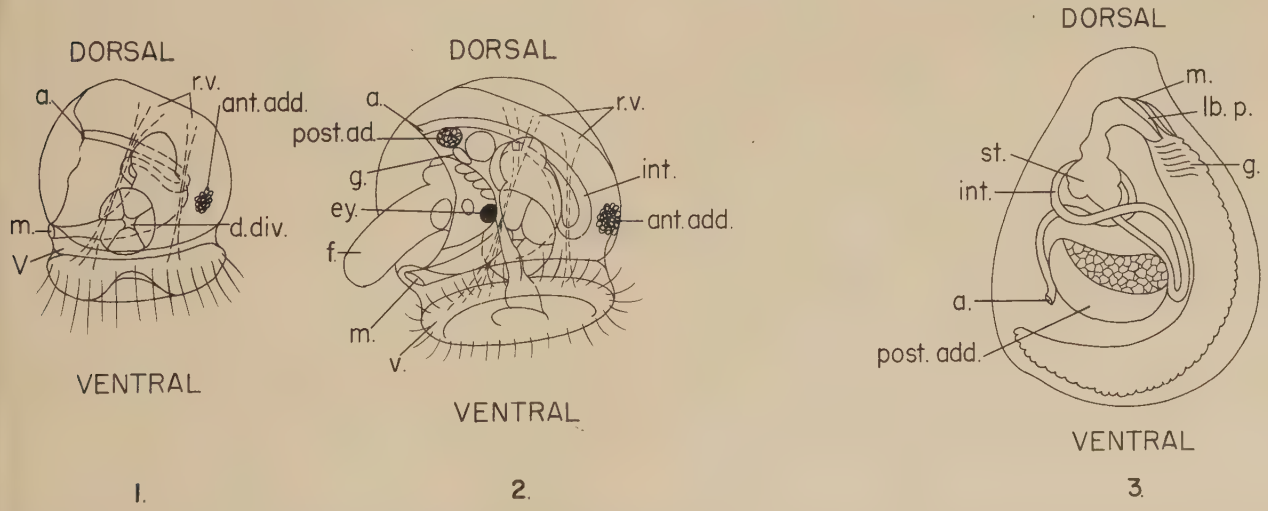

FIGURE 352.—Diagram showing the changes in the topographical relation of various organs of *O. edulis* during the transition from free-swimming larva (1) to fully developed larva ready to set (2) and juvenile oyster (3). From Erdmann (1935). a.—anus; ant. ad.—anterior adductor; ey.—eye; f.—foot; g.—gills; int.—intestine; l.p.—labial palps; post. ad.—posterior adductor; r.v.—retractors of velum; v.—velum.

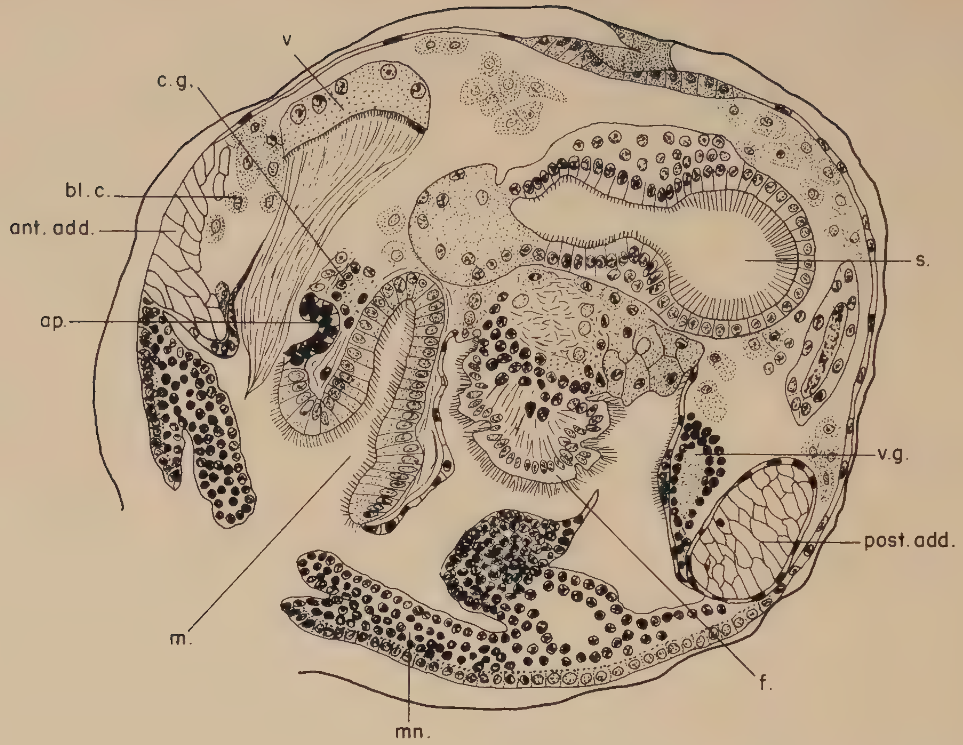

FIGURE 353.—Sagittal section of spat of *O. edulis* about 24 hours after attachment. ant. add.—disintegrating anterior adductor muscle; ap.—apical area of the velum; c.g.—cerebro-pleural ganglion; f.—foot; mn.—mantle; m.—mouth; post. add.—posterior adductor muscle; s.—stomach; v.—velum; v.g.—visceral ganglion. After Cole, 1938b.

of *Dreissensia polymorpha* and unknown to Stafford showed very clearly that the velum of this bivalve disintegrates and is absorbed, and that the apical area comes to lie outside the esophagus and later is fused with the upper lip of the mouth to form the basis of the labial palps. Cole (1938b) showed in a series of sections of *O. edulis* that as the velum collapses almost immediately after setting, its entire structure is moved upward and forward. Most of it is either cast off or disintegrates, and parts of it probably are swallowed. The apical area or apical plate of the velum becomes detached from surrounding tissues and sinks to a position dorsal to the esophagus below the surface of the body (fig. 353, ap.) where it fuses with the upper lip of the mouth. Subsequently the thickened upper lip extends laterally to form the upper labial palps. The cerebro-pleural gangial (c.g.) can be seen underlying the apical plate. In 48 hours all traces of the velum disappear.

Reabsorption of the foot begins after the discharge of the contents of the byssus gland during attachment. The foot gradually shrinks and projects behind the mouth as an irregular mass of tissue covered with ciliated cells. Phagocytes invade the interior of the foot and digest the tissue. The disentegration of the foot of *O. edulis* is completed in about 3 days.

The fully developed oyster larva has two adductor muscles. The posterior muscle, discovered by Jackson (1888, 1890), is not found in the early veliger but appears in the umbo larva. Both muscles are of approximately equal dimensions. Following attachment the anterior muscle degenerates while the posterior moves counterclockwise in the same direction as the mouth and anus.

The eyespots of *O. edulis* break down and disappear after the first 24 hours of attached life. The outlines of the epithelial cup become irregular because it is invaded by phagocytes that ingest the pigmented eye cells, thus causing the liberated pigment to lie in irregular clumps.

Many phases of larval-metamorphosis, especially of the *Crassostrea* group of oysters, are inadequately known and need to be more critically studied. With advances in the technique of artificial rearing of oyster larvae this gap in the knowledge of oyster biology may soon be filled.

DISPERSAL OF LARVAE

During the 2 or 3 weeks of free-swimming life the larvae of *C. virginica* are more or less passively carried by currents and are widely distributed in coastal waters. Biologists who have studied the distribution of planktonic bivalve larvae (Thorson, 1946) agree that their swimming is not strong enough to overcome the water movements which transport them far from the spawning grounds. To a certain extent larvae combat the currents by closing their valves and sinking to the lower level of the water column or to the bottom. However, observations of the swimming habits of artificially raised larvae of *C. virginica* kept in tall containers in the laboratory show that most of them remain swimming nearly all the time, and only those that appear to be too weak or are infected by fungi settle to the bottom.

Various methods are used in oyster research to study the distribution of larvae by taking quantitative samples, but none are satisfactory, and the results obtained by the different methods are not comparable. A pump for pumping measured volumes of water from different depths, plankton tow samplers of various designs, plankton traps, and bottle collectors of the type described by Thorson are the devices used in the study of vertical distribution of oyster larvae. The plankton tow net is most frequently employed. Larvae may be filtered out through screens, or a preserved sample of water may be placed in a glass cylinder with the bottom drawn into a funnel with a drain cock. The water may be centrifuged at high speed using the Foerst type electric centrifuge designed primarily for the collection of minute organisms that ordinarily pass through the finest mesh of the plankton net.

Many observers have found that newly attached young oysters far outnumber the free-swimming larvae, particularly of the umbo stage, found in plankton samples (Prytherch, 1924; Galtsoff, Prytherch, and McMillin, 1930; Loosanoff and Engle, 1940). Similar observations concerning the scarcity of larvae of *O. edulis* were reported by Spärck (1925) for Limfjord waters and by Gaarder (1933) for two Norwegian oyster ponds where the oyster larvae were present only in the deeper and saltier layers of water. Observations on the abundance and distribution of oyster larvae made in this country and abroad have been adequately reviewed by Korringa (1941).

The problem of adequacy of plankton sampling in relation to the physical and chemical hydrology of the James River, Va., oyster seed bed area was investigated by Pritchard (1952, 1953). His calculations show "that the concentration of late stage larvae in the overlying water sufficient to produce the large observed set needs to be, on the average, only about one larva for 100 liters." Since the basic sampling employed in these studies of distribution of oyster larvae was 100 l., the inadequacy of such a sampling technique is obvious and some better automatic sampling methods should be used to clarify these obscure points of larval behavior.

In estuaries the vertical distribution of larvae seems to depend on changes in the velocity and direction of tidal currents and the vertical salinity gradients. The oyster larvae have a more or less uniform vertical distribution in rearing tanks (Cole and Knight-Jones, 1939) and in the estuaries and bays wherever water mixing has prevented the formation of vertical gradients of temperature and salinity.

Several observers have attempted to correlate the distribution of larvae with different stages of tides. Julius Nelson, one of the pioneer students of the biology of the larva of *C. virginica* in New Jersey waters, believed that the larvae could migrate toward land by rising at the beginning of flood tide and settling to the bottom before the turn to the ebb. By this reaction to tidal changes their dispersion in tidal estuaries is avoided. This idea influenced the research of his son, Thurlow Nelson, and his students, who modified and elaborated the original concept (Nelson, 1917, 1921; Nelson and Perkins, 1931; Carriker, 1951).

According to these observations, which were made in New Jersey estuaries, the swarms of larvae are distributed along definite lanes up and down stream from the spawning grounds. If the salinity of water is uniform from bottom to surface, the greatest number of larvae is found at the level of the highest current velocity. In bodies of water with distinct salinity stratification the larvae congregate just above the zone of greatest salinity change. Nelson believed that the advanced larval stages drop to the bottom and remain near it during slack water, and that the increased salinity of early flood tide stimulates their swimming upward. This performance repeated at each change of tide enables the larvae to move upstream by progressive stages. This mechanism, if true, would explain the location of many setting areas in tidal rivers above the principal oyster grounds. The theory has stimulated a great deal of field observation, but, unfortunately, the experimental evidence upon which it rests has not been fully documented. Only a few laboratory observations have been made on the effects of changes in current velocities and salinity on the behavior of oyster larvae, and the experiments reported by Nelson and Perkins were performed under the most primitive conditions. Great experimental difficulties were involved in conducting this type of study, and the elaborate equipment necessary for recording larval behavior was not available to the investigators.

Observations on larval distribution in waters other than New Jersey differ from those described by Nelson and his associates. Prytherch (1929) states that in Milford Harbor, Conn., "the oyster larvae were found to be most abundant at the time of low slack water and gradually disappeared as the tide began to run flood." He further states that no larvae could be found swimming in the water when the flood current had reached a velocity of 0.6 foot per second, and supports this statement by observations on oyster larvae kept in a tank. Oyster larvae remained swimming in the tank while the water was at a standstill, but dropped to the bottom when the current velocity produced by artificial circulation was from 0.3 to 0.5 foot per second. The experimental technique was very primitive, and the results cannot be considered convincing. Observations made by Loosanoff (1949) in Long Island Sound do not confirm Prytherch's interpretations. No evidence was found that early and late umbo larvae were common near the bottom. On the contrary, in several instances "their number was greatest midway between the high and low water when the tidal current was near the maximum velocity." A similar conclusion that larvae do not descend during periods of rapid tidal flow was reached by Carriker (1959) from studies of conditions in a salt-water pond on Gardiners Island at the eastern end of Long Island, N.Y.

Current views of the movement of oyster larvae up estuaries were summarized by Carriker (1961). The consensus of opinions of those who studied the problem in typical estuaries indicates that fully developed larvae (pediveligers) have a tendency to remain in lower, more saline strata and are passively conveyed toward the upper reaches of an estuary by the net, nontidal flow of deeper and denser layers of water (circulation in the estuaries is discussed in Chapter XVIII, p. 402). The discrepancy between the observations made in New Jersey waters and in Long Island Sound may be explained by differences in hydrography. Long Island Sound is not an estuary in the strict meaning of the term, but can be regarded as an embayment with several true estuaries, as for instance, the mouth of the Housatonic River, Milford Harbor, New Haven Harbor, and many others. The distribution of the larvae in the Sound is not, therefore, comparable to that observed in New Jersey waters. Further, salinity change from surface to bottom is small, rarely exceeding 2 °/₀₀, and there is considerable exchange of sea water between the Sound and the outside waters. Under these conditions one may expect substantial losses of larvae during a tidal cycle. It is known that abundance of fully grown larvae in the Sound area is so low that quantitative sampling is not reliable.

The evidence that oyster larvae are actually conveyed by tidal current to the upper part of a tidal river is provided by the investigations of Dimick, Egland, and Long (1941) on *O. lurida* in Yaquina Bay, Oreg. Yaquina Bay and River is a short estuary, about 12 miles, on the coast of Oregon. The natural oyster beds cover only 101.9 acres. Plankton samples taken systematically at known distances from the mouth of the bay showed that "up-river limit of the free-swimming larvae was . . . approximately 4 miles above the upper limits of the natural oyster beds." No larvae were found in this area at near low tide. There is no doubt that these larvae were carried upstream by flood tide.

Lack of agreement on the results of field observations on the relation of larvae to tidal stages is the result of inadequacy of sampling techniques and a lack of understanding the responses of the larva to environmental changes. Changes in temperature, salinity, current velocities, oxygen, and food content of water vary in each estuary, so the occurrence or absence of larvae cannot be related to a given tidal phase unless the major conditions during this stage of tide are fully understood and their effects on larvae are known.

The volume of water transported by ebb flow in estuaries usually exceeds the volume of water re-entering at flood, the difference being equal to the volume of river discharge at the head. If the larvae are uniformly distributed in the water and swim most of the time, a certain percentage of them will be carried away and lost in the sea. Many more are lost as prey to enemies, disease, and other causes.

In the light of present knowledge only two general assumptions regarding the larval behavior can be made: oyster larvae are able to move by their own power within only a very limited area, and they are dispersed by tidal currents beyond the immediate vicinity of spawning grounds.

A survival relationship exists between the age of the larvae and tidal cycles. After analyzing daily counts of larvae of *O. edulis* in plankton samples taken in Oostersheld, Holland, Korringa (1941) concluded that the longer the duration of the pelagic period the greater is the loss of larvae and the lower is the percentage reaching maturity: In 6 to 7 days, equal to 13 tides, 10 percent reach maturity; in 10 days, equal to 19 tides, 5 percent reach maturity; and in 12 days, equal to 23 tides, 2.5 percent reach maturity. If the original number of larvae is A and the rate of dispersal and other losses of larvae are equal during their free-swimming period, the number of larvae at the completion of pelagic life is $A(1-1/p)^n$ where 1/p is the decrease during one tidal cycle and n is the number of tides. The loss during one tidal cycle is estimated by Korringa at between 13 to 15 percent. About 10 percent of the losses he attributed to predators and only about 4 percent to tides. Because of the greater duration of the pelagic life of the oviparous *C. virginica*, it is reasonable to expect that losses of larval populations of this species probably exceed those determined by Korringa for the larviparous *O. edulis*.

It is generally known that mortality among the planktotrophic larvae during their pelagic life is tremendous and that only an insignificant percentage of them reach metamorphosis. Korringa made an interesting computation which shows that out of one million *O. edulis* larvae produced in Oostershelde only about 250 attach themselves and metamorphose, and of this newly set spat 95 percent die before the onset of winter.

The rate of survival of larvae of *C. virginica* and the percentage reaching attachment are not known, but the principles of Korringa's method can be applied to the American species. His studies show that the success of oyster setting depends on prolific and simultaneous spawning of oysters in an estuary. By determining the abundance of larval population and the rate of exchange of water during a tidal cycle, an estimate can be made of the intensity of the forthcoming setting, barring, of course, unforeseen circumstances which may destroy the larvae.

REACTION OF LARVAE
TO EXTERNAL ENVIRONMENT

Little is known about the reactions of larvae to changes in temperature and salinity of water. Temperature fluctuations during the reproductive season apparently have no direct effect on the behavior of larvae of *C. virginica*, *O. edulis*, and *C. gigas*. Davis (1958) has demonstrated in a series of laboratory tests that the reduction of salinity from the normal (for Long Island Sound oysters) level of 26‰ to 27‰ to 15‰ has no effect on the growth of larvae and that inhibition of growth became noticeable in salinities of 12.5‰ and lower (Davis and Ansell, 1962). In water of 10‰ salinity 90 to 95 percent of the larvae died by the 14th day, and at a salinity of 5‰ they appeared to be moribund within 48 hours. In these experiments the behavior of larvae was not recorded. It would be interesting to repeat these studies and determine the reactions of larvae to sudden and to gradual changes of salinities.

Vertical distribution of larvae of *O. edulis* apparently is not affected by light (Korringa, 1941). This is probably true also for the larvae of *C. virginica*, but because no experiments have been made under controlled laboratory conditions, it is premature to assume that larvae of the American oyster are not sensitive to light. The phototactic responses of larvae to light intensity and color have not been explored, but the presence of the eye in the fully developed larva suggests that this organ is somehow used during the last days of larval life. Before attachment the larva crawls over the surface exploring the substratum with its foot, which acts as a tactile organ. It has not been established that the eye participates in this exploration. Nelson (1926) believes, however, that the "eyed" larvae of *C. virginica* are stimulated by light and continue to move until they reach a shaded place where they become quiescent. Hopkins (1937) expresses the opposite view and states that in setting of *O. lurida* light is not an orienting factor. He inclines toward Prytherch's (1934) view that the larval eye has an entirely different function. Since neither of the quoted authors can corroborate their impressions by experimental evidence, the whole question of the factors influencing the behavior of oyster larvae at the time of setting needs to be examined.

TO THE ANGLE OF SURFACE

French oyster growers take advantage of the preference of oyster larvae for the under surfaces of submerged objects and use special spat collectors made of tiers of tiles set one upon the other with their concave surfaces underneath. New spat is always found in larger numbers on the lower surfaces. According to Cole and Knight-Jones (1939) the larvae of *O. edulis* reared in large tanks in Conway, Wales, set more intensely on the under surfaces of test shells. A study of the effect of the angle of a flat surface on the attachment of larvae was made by Hopkins (1935) in his work on *O. lurida* of the Pacific Coast. He used glass plates, each 2,400 sq. in., placed at different angles over the oyster grounds. The under horizontal surface was designated as 0° and the upper horizontal as 180°. Other plates were set at 45° intervals between the two extremes. The average number of larvae attached to each surface were:

0° (under horizontal)	1, 195
45°	181
90°	11
135°	3
180° (upper horizontal)	1

Similar observations were made by Schaefer (1937) with the larvae of *C. gigas*. He set 150 glass plates in positions varying by 45°; some of the plates were parallel to the direction of the tidal current while others were transverse to it. The plates were left for 5 days before the spat were counted. There is a functional relationship between the intensity of setting and the angle of the surface on which the larvae set, the number of larvae being greatest on the under horizontal surface (0° angle) and lowest as the angle approaches 180°. The curve shown in figure 354 is drawn between the points taken as the average numbers of larvae attaching during 5 days on a glass surface held at different angles. The curve is hyperbolic. Schaefer attributes the setting behavior of *C. gigas* to the upward position of the foot of the swimming larvae and possibly to negative geotaxis. No experimental evidence is given to substantiate either point.

The behavior of oyster larvae does not differ from that of many other fouling organisms which were found by Pomerat and Reiner (1942) to attach in greatest abundance to the under surfaces of plates held in a horizontal position.

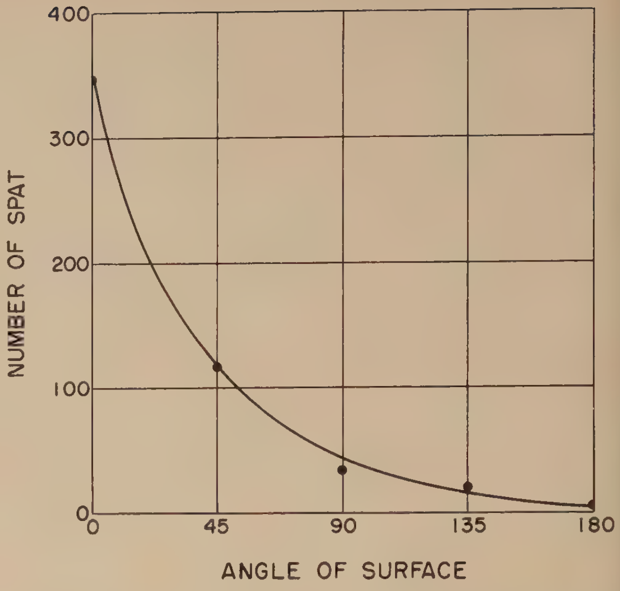

FIGURE 354.—Effect of angle of the surface of plate glass on the number of spat of *C. virginica* which attach to it within 5 days. Glass area 2,400 sq. in.; 0° is under horizontal surface. (Figure 1 from Schaefer, 1937).

Contradictory results were obtained, however, in experiments with cement covered boards held at several angles either suspended in water or placed near the bottom. These tests made by Butler (1955) on oyster bottoms near Pensacola, Fla., showed a preponderence of spat on the upper surfaces of the boards. Setting on upper surfaces (135° and 180°) comprised 78 percent of the total number set, and only 18 percent were counted on the lower surfaces. The remaining 4 percent were found on vertical boards. Butler was not able to confirm the results of Pomerat's and Riener's tests made earlier at Pensacola in which frosted glass plates suspended in water attracted the greater percentage of oysters (and barnacles) to the lower surfaces. Bonnot (1937, 1940) built a spat collector from plywood strips dipped in a mixture of cement and sand. He fastened five or six strips of plywood one above the other so that the horizontal surfaces were separated by three-fourths of an inch. Setting of *O. lurida* averaged 98 larva per square inch on the upper surfaces and 67 per square inch on the lower ones. The turbulent current caused by narrow spaces between the strips possibly was responsible for the greater number of larvae setting on the upper surfaces.

A lack of consistency in observations of various investigators in different environments indicates that it is impossible to ascertain the effects of a single factor of the environment while testing under complex and variable natural conditions. Real progress in the study of the reaction of oyster larvae may be achieved if further observations are made under controlled conditions.

Larvae of *C. virginica* that are grown artificially in culture jars and not disturbed by stirring or aeration are more or less uniformly distributed. Eyed larvae frequently congregate on the surface, swimming with their vela uppermost and touching one onother with the tips of the cilia. They form groups or "rafts" visible to the naked eye. Some of them close their valves, fall rapidly to the bottom, and after a short time resume swimming. Falling to the bottom should not be confused with negative geotaxis, which has not been demonstrated for oyster larva. In the laboratory, larvae often attach themselves to the sides of plastic or glass containers and apparently do not discriminate between light and dark surfaces.

TO THE PROPERTIES OF SURFACE

Oyster larvae attach themselves to many kinds of hard and semihard surfaces. They are found on rocks, gravel, cement, wood, shells of other mollusks, on stems and leaves of marsh grass, and on a great variety of miscellaneous objects such as tin cans, rubber boots and tires, glass, tar paper, and pieces of plastic that may be accidentally thrown on the bottom or deliberately used as spat collectors. There is no evidence that the larvae are selective in finding a suitable place to set, provided the surface is not covered with a slimy film, detritus, or soft mud. Under natural conditions they are never found on shifting sand or on a bottom covered with loose sediment. Success of setting always depends primarily on the availability of clean surfaces rather than on other factors. Shells covered with oil and greasy substances in polluted areas are not suitable for the attachment of larvae. Cole and Knight-Jones (1939) found that it is difficult to induce *O. edulis* to set on smooth glass, but the larvae of *C. virginica* raised in the laboratory readily attach to polished glass. In fact live preparations of spat may be obtained for microscopic examination of small oysters by suspending glass slides in a tank with fully grown larvae.

GREGARIOUSNESS

An interesting gregarious tendency has been observed by Cole and Knight-Jones (1949) among the larvae of *O. edulis*. During experiments in large rearing tanks they found that larvae set more readily on shells already bearing 50 to 100 spat than on shells bearing fewer spat. They suggest that a substance secreted into the surrounding water by the spat, and possibly by the fully developed larvae, encourages the setting. No attempts were made to isolate the substance and test its effect. The authors make another observation which may throw some doubt on the validity of their interpretation. They state (p. 36) that "Larvae set more readily on shells which had remained uncleaned in the tanks for 2 or more weeks, and which bore a visible film of bacteria or diatoms, than on similar shells which were cleaned daily." In their study of gregariousness they placed shells of uniform size and shape in pairs in a tank containing fully developed larvae, and the number of spat attached to them was counted daily. One shell of the pair was considered a control and was cleaned every day, and the other (experimental) remained uncleaned. By the end of the setting period the total spat settled on the experimental shells significantly exceeded the total spat settled on the controls by a ratio of 2.5 to 1. The figures suggest that the observed differences may be due to the attraction of larvae by those which had already settled on the shell, but the conclusion cannot be accepted without further verification. The possibility is not excluded that some other unknown factor, such as the position of the controls in relation to the experimentals, affected the results or that handling and removal of spat from the control shells caused changes to the surface which made them less attractive to the larvae. It would be profitable to conduct a series of tests designed to eliminate bias by placing experimental and control shells at random and making a statistical analysis of the significance of the differences.

Yonge (1960) expresses no doubt "that larvae (of *O. edulis*) settle more readily on surfaces to which others are already attached," and points out that this tendency aids in reproductive efficiency and is, therefore, a major benefit to attached animals. In view of the fact that a single oyster shell has sufficient space for only a few spat to grow to maturity, heavy concentrations of spat are of doubtful value for reproduction and may even be harmful by creating overcrowded conditions.

ARTIFICIAL REARING OF OYSTER LARVAE

Early attempts to rear oyster larvae under artificial conditions produced uncertain results. Sometimes a small number of spat were obtained, but the experiments could not be repeated under similar conditions. At that time oyster larvae were placed in 5-gallon carboys, and the water was aerated and circulated. At 2-day intervals the larvae were concentrated by centrifuging and transferred into fresh sea water (Wells, 1920). In another method, tried with only partial success, the larvae were reared in slowly running sea water which was filtered through a 2-inch layer of white sand or porous stone (filtrose) placed on the bottom of a container. The rates of filtration and of addition of new water were regulated by a valve placed below the filtering layer (Prytherch, 1924). In both types of experiments no food was added to the containers under the assumption that enough was present in the water. Then Gaarder and Spärck (1933) and Gaarder (1933) studied the food of the larva of *O. edulis* in Norwegian oyster ponds and made what may be considered the first significant step toward solving the problem of rearing larvae under artificial conditions. Spärck observed that the water of the ponds contained considerable numbers of a small green unicellular alga which later on was isolated and cultured in the laboratory. It appeared to be a species of *Chlorella* which was consumed by the larvae. Studies by these investigators revealed also that nannoplankton of the ponds consisted principally of small green algae and flagellates measuring from 2μ to 3μ. Fertilization of experimental tanks by the addition of liquid manure greatly increased the production not only of *Chlorella* but also of various diatoms, chiefly *Nitzschia*, flagellates, various large unicellular green algae, and bacteria. In this enriched water a few larvae grew to a size of 300μ but failed to attach (Spärck, 1927). After it was found that *Chlorella* is present in the Norwegian oyster ponds, in experimental tanks in Conway, Wales, and in certain experimental basins in Denmark, Kändler (1933) attempted to grow oyster larvae on a diet of this alga alone but had little success. This led him to conclude that oyster larvae are unable to digest *Chlorella*, which left the intestine apparently unchanged. Feeding experiments with *Carteria* and *Chlamydomonas* were also unsuccessful. More critical experiments conducted at Conway, Wales, showed that the larvae are unable to utilize nonmotile green algae such as *Chlorella* and *Collomyxa* but that yellow-brown chrysomonads (not identified but designated as flagellate C) gave satisfactory results (Cole, 1937).

The Conway experiments demonstrated that organic enrichment of the water of the large tanks was consistently successful in giving rise to a good crop of flagellates with the resulting good growth and setting of larvae (Cole, 1939). The most satisfactory fertilizer was the meat of the shore crab *Carcinus* ground with sand and heated to the boiling point. The suspension of meat was added to a 90,000-gallon tank at the average rate corresponding to 12.5 medium-sized crabs per day for a period of 3 to 4 weeks. Production of nannoplankton was judged by pH readings, and as soon as the readings reached 8.3 to 8.4 and the tank had a distinct slight cloudiness, no more crab meat was added.

Evidence presented by Cole showed that growth and attachment of *O. edulis* larvae in tanks were significantly increased by organic enrichment which stimulated the development of the nannoplankton. Under laboratory conditions the oyster larvae grew and set satisfactorily in the water containing cultures of *Platymonas tetrahele*. The larvae of oysters and other bivalves apparently are not able to swallow microorganisms which exceed 8μ, but according to Thorson (1950) the size of nannoplankton normally devoured by larval forms is smaller (2μ to 3μ).

Difficulties in obtaining reproducible results from using organic enrichment for rearing larvae suggested that variations in the composition and quantity of nannoplankton may be responsible. To determine the food requirements of *O. edulis* larvae, Bruce, Knight, and Parke (1940) isolated from sea water six flagellate organisms ranging in size from 1.5μ to 7μ in diameter. A known number of oyster larvae were introduced into glass vessels filled with 16 l. of uncontaminated, sterile sea water which was stirred and aerated. The water was changed continuously by a drop feed; the loss of larvae was prevented by covering the outflow tubes with bolting silk. The larvae were fed pure cultures of flagellates grown in the so-called "Erdschreiber" medium of the following composition (Gross, 1937):

Sodium nitrate ($NaNO_3$)	0.1 g.
Sodium orthophosphate (Na_2HPO_4)	0.02 g.
Soil extract	50 ml.
Sea water	1,000 ml.

Soil extract is made by boiling 1 kg. of good potting or garden soil with 1 l. of distilled water in an autoclave for 1 hour. The flask is set aside for 2 or 3 days, and the muddy dark fluid is decanted and sterilized by heating to the boiling point. After standing 3 to 4 weeks the suspended particles settle on the bottom, and the transparent brown or red fluid is poured into another container and boiled for a short time. Boiling of the medium should be avoided once the required quantities of nitrates and phosphate have been added.

Since the six flagellates used in these experiments (Bruce, Knight, and Parke, 1940) were not identified and were labeled only by letters, inconsistencies in the results reported may be attributed to the appearance in the culture of other species, or, as the authors state, "to the supervention of factors outside experimental control." The authors suggest that one of these conditions may be the fact that larvae from different oysters are not equally viable.

The feeding of oyster larvae (*O. edulis*) with pure cultures of nannoplankton was repeated by Walne (1956). In this case the larvae were kept in vessels of 1 l. capacity without change of sea water, and the species of flagellates grown in cultures were identified. Among the Chlorophyceae, only *Pyramimonas grossii* Parke gave consistently good results. Tests made with *Chlorella stigmatophora* Butcher seemed to indicate that those chlorococcales which have a thick cell wall are poor food for oyster larvae. The best results were obtained with *Isochrysis galbana* Parke, a chrysophycean of about 5μ to 6μ in length. *Prymnesium parvum* Carter was found to be toxic to larvae. So far there is no proof that the species of flagellates used in these experiments form a significant component of the natural population of nannoplankton and that their presence in estuaries is necessary for larvae living under natural conditions.

Imai and Hatanaka (1949, 1950) reported that the larvae of *C. gigas* can be reared on a culture of colorless flagellate, *Monas* sp., which abounds in brackish waters of Japan. The authors believe that the flagellate of the *Monas* type plays an important role in the production of oysters in Japan. The possibility remains, however, that in their experiments other flagellates were present in the culture of *Monas* enriched with glucose, cane sugar, nitrates, and phosphates.

The pelagic life of *C. virginica* and *C. gigas*, and probably of all oviparous oysters, is longer than that of larviparous *O. edulis* and *O. lurida*. Consequently, the rearing of these oviparous larvae under artificial conditions presents additional difficulties. Considerable advances in the rearing of larvae of various bivalve species were made by Loosanoff, Davis, and their collaborators at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn. Phases of the work are summarized by Loosanoff (1954) and Loosanoff and Davis (1963a, 1963b). Oysters were induced to spawn by increasing the temperature and by adding sperm suspension (see p. 308, Chapter XIV). The fertilized eggs were freed from debris by passing the water through a series of fine screens and placed in 5-gallon earthenware jars until free-swimming larvae emerged. Then the water was changed every 24 to 48 hours by straining it through fine sieves which retained the larvae. The sea water in which the larvae lived was filtered through cotton to remove detritus and zooplankton. Aeration and mechanical agitation were considered unnecessary if the water was changed every other day. The larvae were given measured amounts of cultures of various micro-organisms. In general the results obtained in Milford corroborate the findings of British investigators. Davis (1953) established that oyster larvae can utilize as food the following species of flagellates: *Dicrateria inornata*, *Chromulina pleiades*, *Isochrysis galbana*, *Hemiselmis rufescens*, and *Pyramimonas grossii*. *Chlorella* sp. was used only by advanced larval stages and not by young veligers.

The utilizable flagellates were added to the rearing tanks at the rates of 15,000 and 25,000 cells per ml. per day but no toxic effects were noticed in these heavy concentrations, and the larval oyster population of approximately 5,000 per l. showed satisfactory growth. The actual number of flagellates ingested by the larvae was not determined, but the inference was made that "the rate of growth of oyster larvae had an inverse relation to the number of larvae per unit volume" (Davis, 1953). Cole (1939) states that a population of 20,000 to 30,000 small flagellates per 1 ml.

is adequate to promote growth of larvae of *O. edulis* but is insufficient for the spat.

With the exception of the toxic *Prymnesium parvum*, the naked flagellates provided better food for young oyster larvae than the organisms with heavy cell walls, which can be utilized only by older larvae. The best single foods were found to be *Isochrysis galbana*, *Monochrysis lutheri*, *Chromulina pleiades*, *Dicrateria inornata*, and some other unidentified species of *Dicrateria*. Since the cultures used in these experiments were not free of bacteria, the question naturally arises whether the marine bacteria are utilized as food. Davis (1953) states that none of the 13 species of marine bacteria tested by him were used by the larvae. The species of bacteria have not been identified. However, the probability that larvae may derive a certain amount of food from some bacteria is strengthened by the observation reported by Davis (1953) and Loosanoff (1954) that larvae kept in cotton-filtered sea water without algal food continued to grow for as long as 14 to 18 days. The role of marine bacteria in the feeding of oyster larvae needs further experimental study.

Apparently the best results in rearing larvae under artificial conditions are obtained with a mixed food of *Isochrysis galbana*, *Monochrysis lutheri*, *Chromulina pleiades*, and *Dicrateria* sp. With such a diet and at 30° C., the larvae of *C. virginica* begin setting between the 10th and 12th days after fertilization; at 24° C., the sibling larvae are ready to set on the 24th to 26th day; at 20° C., only a few of the larvae set by the 38th day. Setting of larvae of *O. lurida* at a temperature of 22° C. takes place on the 7th day after release of larvae from the brood chamber (Loosanoff and Davis, 1963a).

Under laboratory conditions in Woods Hole the young larvae of *C. virginica* are often found on the bottom of vessels entangled in lumps of several individuals. These larvae never recover and usually die within the next 24 hours. Sometimes the larvae of oysters and clams are attacked and killed by a fungus which has been tentatively identified by the workers at the Bureau of Commercial Fisheries Biological Laboratory at Milford, Conn., as belonging to the genus *Sirolpidium zoophtorum* Vishniac (Davis, Loosanoff, Weston, and Martin, 1954; Johnson and Sparrow, 1961). There are undoubtedly other bacteria and possibly viruses which inflict epizootic mortality on larval populations in the laboratory and in natural waters.

The technique of rearing oyster larvae has progressed sufficiently to be applicable to practical purposes of oyster culture. Details of techniques, organization, and operation of a mollusk hatchery are summarized by Loosanoff and Davis (1963b).

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CHAPTER XVII

CHEMICAL COMPOSITION

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Regardless of the zoological group to which an animal belongs the greatest mass of materials which form the tissues and organs, exclusive of skeleton or shells, consists of three major groups of organic compounds: proteins, carbohydrates, and lipids (fats). Many analyses reported in the literature show that, in spite of great variability in the composition of meat of several species of *Ostrea* and *Crassostrea*, the order of magnitude of the three components is common to all the species studied. The proteins make up 50 percent or more of the solids, carbohydrates are less than 25 percent, lipids constitute less than 20 percent.

PROXIMATE COMPOSITION OF OYSTER MEAT

A general idea of the proximate composition of the meat of *C. virginica* can be deduced from tables published by the U.S. Department of Agriculture for dietitians, nutritionists, physicians, and others engaged in planning diets or in calculating the nutritive value of foods (Watt and Merrill, 1950, p. 36). The material used for such analyses represents the average sample available for purchase at the market or delicatessen store. The figures do not refer, therefore, to oysters of any particular locality or to time of the year. For convenience in making a comparison all the values originally given for 1 cup (240 g.) of raw oysters were recomputed for 100 g., which corresponds to five to eight medium-size oysters. The sample contained 9.8 g. of protein, 5.6 g. of carbohydrates, 2.1 g. of fat, 94.1 mg. of calcium, 143 mg. of phosphorus, and 5.6 mg. of iron, and 80.5 g. of water.

When oysters are prepared for the market the meats are shucked and washed, either in fresh water or sea water. During this process the water is stirred and air is blown through it to remove grit, pieces of broken shell, and mud. The procedure affects the chemical composition because some of the soluble salts present in the body are lost, and the less soluble constituents, the proteins and fats, then make up the greater proportion of solids. Consequently the values for these two components quoted above are somewhat higher than for unwashed oysters. Correspondingly the values of mineral salts in Watt and Merrill's data are lower.

SEASONAL AND LOCAL VARIATIONS

Variations in the chemical composition of oysters follow distinct patterns related to environment and season of the year. The major environmental factor affecting chemical composition is the salinity of water. *C. virginica* is an estuarine species which may be found in waters ranging from almost $40\,^{\circ}/_{\circ\circ}$, as in the sheltered bayous of the Gulf Coast, to less than $3\,^{\circ}/_{\circ\circ}$ at the upper reaches of bays after heavy rainfall (upper Chesapeake Bay, Mobile Bay, Apalachicola Bay, and others). A change from wet to dry spells produces a pattern of fluctuations in the contents of mineral salts in oysters growing in waters of fluctuating salinity. Such conditions prevail in the waters of the south Atlantic and Gulf states where the annual range of changes from maximum to minimum ash content was reported to be 5.3 to 31.1 percent on a moisture-free basis. The solids, for the same period of time, varied between 7.5 and 18.4 percent of the wet weight of oysters (Lee, Kurtzman, and Pepper, 1960). Fluctuations in the moisture content due to absorption of water and loss of solids are the most significant features of changes in the chemical composition of oyster meat which affect their commercial quality. Good oysters contain two and one-half times more solids per unit of volume or weight, and obviously have higher nutritive value than the poor ones containing over 92.5 percent water.

Oysters living under marginal conditions (see chapter XVIII) are usually low in solids throughout the year. A comparison of the mean annual composition of meat based on a series of regular observations discloses these differences. Table 38 summarizes the chemical studies made for 2 consecutive years on oysters from six southern states (Lee and Pepper, 1956; Lee, Kurtzman, and Pepper, 1960). The lowest values of total solids, and of proteins, carbohydrates, and fat were found in Georgia and the highest in Louisiana and Alabama oysters. Data on seasonal variation in the composition of meat for the southern oysters were analyzed by Lee and Pepper (1956). Solids increase steadily from 9.5 percent in October to about 13.5 percent in March; in the middle of May they begin to decline and reach the lowest value of 9.2 percent in September. The fat content followed the trend approximately. The changes are associated with the gonad development and spawning, which in the southern oysters begins earlier and continues longer than in oysters of the northern waters.

Table 38.—*Proximate mean composition of meat of* C. virginica *from southern waters for 2 consecutive years, from October 1954 to October 1956 inclusive, in percent of their net weight*

[Recalculated from the data published by C. F. Lee and L. Pepper (1956) and C. F. Lee, C. H. Kurtzman, and L. Pepper (1960)]

State	Solids		Protein		Carbohydrate		Fat		Ash	
	1st year	2d year	1st year	2d year	1st year	2d year	1st year	2d year	1st year	2d year
Louisiana	11.5	12.2	5.8	6.3	2.7	3.0	1.1	1.3	1.9	1.5
Mississippi	11.2	12.5	5.1	6.1	3.0	3.3	1.1	1.6	2.0	1.5
Alabama	11.8	13.8	5.8	6.1	3.3	4.6	1.3	1.6	1.4	1.4
Florida	10.8	11.7	5.0	5.6	2.5	2.9	0.8	1.1	2.5	2.1
Georgia	10.7	10.0	5.0	4.4	2.3	2.1	0.7	1.0	2.7	2.5
South Carolina	13.5	13.9	6.2	6.6	3.7	4.0	1.0	1.1	2.6	2.9

YIELD AND QUALITY OF MEAT

Some idea of geographical differences in the productiveness of oyster bottoms may be gained by comparing the yield of oysters in pounds of meat per bushel. It can be seen from table 39 copied from Power (1962) that the recorded yield of market oysters in the waters of Delaware and further north varies from 6.6 to 7.5 pounds per standard bushel and is significantly higher than in the southern states, from Maryland to Texas, in which the yield is from 3.15 to 5.07.

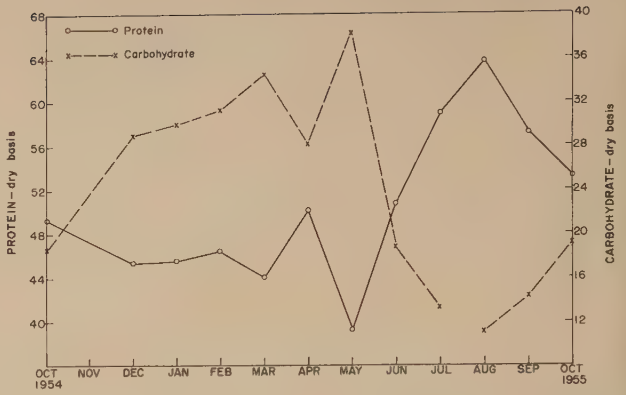

Figure 355.—Average protein and carbohydrate content in monthly samples of southern oysters in percent of dry weight. Oysters for analysis were collected at the shucking plant but were not subjected to the routine washing and air bubbling procedure which causes large salt and fluid losses. From Lee and Pepper, 1956.

The quality of oyster meat is related primarily to the amounts of protein and carbohydrates. The ratio between the two components changes with the season and reproductive cycle. The percentage of protein sharply decreases in May to less than 40 percent of the dry weight while at the same time the carbohydrates reach their maximum of about 60 percent (fig. 355). The actual changes in the protein content are less pronounced because of the increase in solids due to storage of glycogen.

Decline in the ash (mineral matter) content of oyster meat from the highest value of almost 25 percent (dry weight basis) in October to about 5 percent in May (fig. 356) and a gradual increase during May to September are probably related to changes in the salinity of water from which the

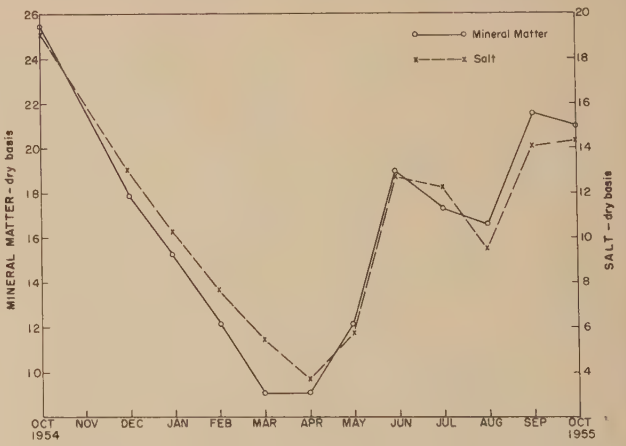

Figure 356.—Average mineral matter and salt content in the monthly samples of southern oysters (unwashed) in percent of dry weight. From Lee and Pepper, 1956.

TABLE 39.—*Yield of market oysters 1960, pounds of meat in U.S. standard bushel*

[From Power, 1962]

Maine	7.50	North Carolina	4.21
Massachusetts	6.50	South Carolina	2.93
Rhode Island	7.00	Georgia	3.15
Connecticut	7.70	Florida, east coast	4.20
New York	7.50	Florida, west coast	4.22
New Jersey	7.01	Alabama	4.17
Delaware	6.60	Mississippi	3.86
Maryland	4.58	Louisiana	4.54
Virginia	4.19	Texas	5.07

oysters were taken, but the problem requires further study.

Variations in the chemical composition of the meat of *O. edulis* are similar to those which take place in *C. virginica*. Gaarder and Alvasker (1941) give a detailed account of these changes in the oysters of Norwegian waters. The extent of annual fluctuations that took place in 1936 are given in table 40.

TABLE 40.—*Extent of changes in the chemical composition of* Ostrea edulis *in Norway in percentage of wet weight*

[According to Gaarder and Alvasker, 1941]

	Weight of meat	Moisture	Protein	Carbohydrate	Glycogen	Fat	Ash
	g.						
Maximum	14.6	81.9	11.2	9.6	7.9	2.5	1.5
Minimum	7.6	76.0	8.8	6.3	5.1	1.6	1.2
Average for 1936	11.3	78.7	10.0	7.8	6.8	2.2	1.3

In a comparison with the analysis of *C. virginica*, the *O. edulis* has a relatively higher carbohydrate-protein ratio and higher fat content. This may be due primarily to the fact that European oysters are grown on oyster farms while the sample of southern American oysters was taken from wild populations. Likewise, the higher yields of *C. virginica* in the waters of northern latitudes is primarily the result of skill in cultivation by private oyster growers of New England and the North Atlantic states rather than geography.

INORGANIC CONSTITUENTS

The mineral content of the edible portion of the oyster consists primarily of sodium chloride (fig. 356), but it also contains almost every chemical element present in sea water. Spectrographic analysis of 22 samples of oyster ash (exclusive of shell) made by the U.S. Bureau of Mines in 1940 at the request of the Bureau of Fisheries (data on file in the library of the Bureau of Commercial Fisheries Biological Laboratory, Woods Hole, Massachusetts) showed that the samples consisted mainly of sodium, potassium, calcium, magnesium, and phosphorus; and low concentrations of the following elements: copper, iron, silicone, aluminum, strontium, lithium, rubidium, nickel, silver, titanium, zinc, vanadium, platinum, manganese, gold, and zirconium. The results are predictable since sea water which contains these elements enters the composition of the oyster's body fluids.

IODINE

The presence of iodine in various sea food animals has been generally known for a long time and has been studied primarily from the point of view of dietitians and nutritionists. Coulson (1934) found that one average serving of *C. virginica* (110 g.) would furnish to the diet 54 μg. of iodine, an amount higher than that found in one serving of red salmon, milk, various vegetables, and beef. The iodine content of fresh oysters handled in the usual commercial manner varied from 1,000 to 11,530 parts per billion on the dry basis, or from 194 to 1,652 parts per billion on the original wet weight basis. When the means of individual variation are considered statistically, there appears to be no significant variation in the iodine content of oysters from different Atlantic and Gulf states nor any significant variation with season. There is, however, a significant difference between the Atlantic and Pacific Coast oysters, (*C. virginica* and *O. lurida*): the Pacific species have a lower iodine content than the Atlantic species. The mode of accumulation of iodine in the oyster tissue and the role it plays in the physiology of the oyster are not known.

The iodine content in oyster meat can be artificially increased by placing live oysters in sea water to which free iodine has been added. In experiments with *C. angulata* at Arcachon, France, Loubatié (1931) showed that the concentration of iodine in the tissues of oysters increased 700 times over its normal value after live oysters were kept for 4 days in water containing up to 3 mg./l. of free iodine. In 1932 a commercial concern at Bordeaux, France, artificially produced such "super-iodized" oysters and advertised their beneficial effect in cases of anemia and other maladies attributed to iodine deficiency. When I visited Arcachon in 1932 there was apparently a good demand for these oysters, which had a strong iodine flavor.

HEAVY METALS

The ability to accumulate various elements present in sea water at very low concentrations is common to many marine invertebrates. Of particular interest is the ability of many bivalves to accumulate various heavy metals, such as zinc, copper, iron, manganese, lead, and arsenic. The problem is of importance because in polluted coastal waters shellfish may store substances that may be dangerous to human health. Hunter and Harrison (1928) showed that oysters affected by industrial pollution in certain coastal areas in Connecticut, New York, and New Jersey contained traces of lead (determined as Pb) and arsenic (As_2O_3), the concentration of the arsenic varying, depending on locality, from 0.6 mg./kg. to 3.0 mg./kg. of dry weight.

The accumulation of copper causes green discoloration of the mantle and gills of oysters and gives them an unpleasant coppery flavor. The problem of greening has attracted many investigators, especially since Lankester (1886) demonstrated that green color in some oysters may be due to an excess of copper, while in the green-gilled European oysters of the west coast of France the bluish-green coloration was caused by absorption of a pigment from a diatom, *Nitzschia ostrearia*, called marenin (Ranson, 1927). Green oysters similar to those of Marennes, France, occur occasionally along the Atlantic coast in Virginia (Mitchell and Barney, 1917) and in North Carolina (personal observation). Accumulation of iron, zinc, and manganese does not change the color of oyster meat.

The degree of concentration of heavy metals in the oyster body is related to the environment. Oysters from the North Atlantic States are poorer in iron and richer in copper than oysters of the South Atlantic and Gulf States in which the relation is reversed. This has been shown by Coulson, Levine, and Remington (1932), who analyzed a number of samples collected from various states in April and again in November-December, 1931. Their observations are summarized in table 41. The data show that the iron content of oyster meat significantly increases from north to south while the copper content decreases. The samples show no significant variations in the manganese content. The increase in iron content is associated with a greater percentage of iron (as Fe_2O_3) in the river water of the South Atlantic States discharged into the estuaries than is present in the runoff waters of the North Atlantic States. High copper content in the oysters of New Jersey, New York, Connecticut, and Rhode Island is possibly associated with the discharge of chemical wastes from shore installations of these highly industrialized states.

OBSERVATIONS ON NEW ENGLAND OYSTERS

Seasonal changes in the composition of oysters can best be studied by regularly taking samples from a single bed containing a population of oysters of known age. Such an investigation was made by taking samples of oysters from a commercial bed in Long Island Sound, off Charles Island, and simultaneously recording the temperature, salinity, and pH of the water. The work was conducted from the Bureau of Commercial Fisheries Biological Laboratories at Woods Hole and Milford. For experimental purposes and for checking analytical methods a large number of 4- to 5-year-old oysters were kept in the outdoor tanks near the laboratories. Samples of 25 oysters were taken once or twice a month for a period of 22 months from July 1933 to August 1935. Ten of the oysters were used for a chemical analysis of ash, 10 for the extraction of glycogen, and 5 for biological studies.

ANALYTICAL PROCEDURES

Oyster meats being prepared for chemical analyses are easily contaminated with iron while they are being removed from the shell. We found that the following analytical procedure was most satisfactory. The surface of the shells was cleaned with a stiff nonmetal brush, and the whole oysters

TABLE 41.—*Iron, copper, and manganese content of oysters from the Atlantic and Gulf coasts*

[Results are expressed in mg./kg. (wet basis). From Coulson, Levine, and Remington, 1932]

Locality		Spring samples			Winter samples		
		Iron	Copper	Manganese	Iron	Copper	Manganese
North Atlantic States	Range	24.9– 32.1	41.2–122.9	1.05–3.00	31.5– 47.1	34.4–137.2	1.56–2.82
	Average	28.9± 1.1	71.8± 6.6	2.09±0.20	40.7± 1.1	85.2± 9.1	2.47±0.1
South Atlantic States	Range	50.0–104.5	4.6– 38.0	1.26–4.16	45.1–135.3	3.4– 36.9	2.0–2.87
	Average	66.0± 8.0	16.1± 3.8	2.49±0.25	70.3± 8.1	17.0± 3.0	2.57+0.08
Gulf States	Range	37.5– 74.8	5.9– 26.8	2.93–4.09	65.2–113.8	19.1– 48.2	3.07–4.40
	Average	59.8± 3.4	16.0± 1.9	3.50±0.15	82.5± 5.0	26.7± 3.0	3.77+0.13

were put in glass containers and placed in an oven at 50° C. for about 1 hour. The meats were then removed with a glass spatula from the gaping valves. The shell liquor remaining in the containers was added to the meats, and a sample of 10 oysters was weighed and placed in a porcelain dish for drying at 90° C. to a constant weight.

The dried samples were pulverized in a glass mortar. Then several grams of the powdered and well-mixed sample were weighed into a silica dish, charred over a low flame, and ashed in an electric muffle at a temperature of 500° C. for 3 to 4 hours. After cooling the sample was moistened with water and 1 ml. of concentrated nitric acid was added. The sample was evaporated to dryness on a hot plate and returned to the muffle, this time at a temperature of 400° C. One application of nitric acid was usually sufficient to complete ashing. Ash was dissolved by heating in a 1:1 solution of hydrochloric acid, 10 drops of hydrogen peroxide were added, and heat was applied until the liberation of oxygen ceased. Finally the sample was weighed, transferred to a 100-ml. volumetric flask and diluted to the 100 ml. mark.

For iron determination Kennedy's colorimetric method of potassium thiocyanate was employed (Kennedy, 1927), using ferrous ammonium sulfate (dried to constant weight) as standard. Copper was determined by Biazzo's method as described by Elvehjem and Lindow (1929) and Elvehjem and Hart (1931). Zinc was determined by Birckner's method, using a nephelometer for comparison of the turbidity of samples (Birckner, 1919) and zinc oxide solution in hydrochloric acid as the standard. Manganese was found by Richards' method (Richards, 1930). To determine the reliability of analytical procedures, several analyses were made in duplicate and occasionally known quantities of metal salts were added to the samples and recovered. In this way error due to analytical procedures was found to vary between 0.5 and 2.5 percent.

Differences in the results of analyses of oyster meat often are due to the method of obtaining samples. The percentage of solids in a sample and the corresponding figure of moisture content depend on the method of drying. Sometimes the sample is dried on a steam bath at a temperature of 90° C.; in other cases the oyster meat is kept in an electric oven at 95° or 97° C. The results will also differ if the sample is first homogenized or if the whole oyster is used for drying.

The main source of inconsistency in the analyses results from methods of discarding the fluid retained in the mantle cavity and in the water tubes and chambers of the gills. This fluid consists primarily of sea water with some blood cells and excretion from the kidney. Oysters removed from the shell with no injury to the mantle and pericardium nevertheless continue to lose blood from the severed ends of the muscle and from blood sinuses in the body proper. The loss of body fluid is very rapid during the first half hour after removal from the shell. For as long as 2 hours after shucking the oyster may lose a quantity of fluids equivalent to 26 percent of the original body weight (Fingerman and Fairbanks, 1956a, 1956b). Puncturing the mantle and pericardium results in up to 50-percent loss of body weight.

To minimize losses of weight caused by prolonged bleeding, oyster meats may be placed on a screen and drained for 5 minutes. More consistent results are obtained if the water captured between the organs is discarded. If the valves are forced apart slightly and jammed open by a small wooden wedge, shaking the oyster with 10 sharp jerks is sufficient to dislodge the water from the gills. This method gives a lower percent of solid content than those obtained with other procedures, because bleeding is minimized.

The percent of moisture in the meat is usually determined by the difference between the total and dry weight of the sample. Direct determination of water content can be made by distillation in xylene in a flask with a reflux condenser. The sample is boiled continuously for 1 hour at a rate of approximately 5 ml. of reflux per minute and for 3 hours at double that rate. Without interrupting the boiling, two drops of 95 percent ethanol are added through the top of the condenser. After the violent ebullations have ceased, boiling is continued for 5 minutes (Calderwood and Piechowski, 1937), then the volume of water accumulated in the side arm of the condenser is measured.

The glycogen content of oyster tissues is determined by digesting them in 30 percent sodium hydroxide for 1 hour at 80° C. Glycogen is precipitated by 95 percent ethanol, washed, dissolved in hot water, hydrolyzed with hydrochloric acid for at least 4 hours at 92° C. and the dextrose present determined in aliquot sample by use of the Hagedorn-Jensen procedure. Details modifying the method to make it suitable for obtaining glycogen in a high state of purity from oyster tissues are given by Calderwood and Armstrong (1941).

VARIATIONS IN GLYCOGEN CONTENT

Glycogen is the reserve material of the oyster. It is stored primarily in the connective tissue of the mantle and labial palps. During the rapid proliferation of sex cells the reserve supply is used, and by the end of the reproductive cycle the amount of glycogen is at a minimum and the mantle is reduced from a thick heavy layer to a thin transparent membrane. Soon after spawning the oysters begin to form and store glycogen and, in the parlance of oyster growers, become fat. The expression fatness as it is used in trade is a misnomer because it does not refer to an increase in lipids. In New England waters the accumulation of glycogen reaches its maximum during late autumn but sometimes continues even in winter. As a rule the glycogen remains at a high level until the beginning of rapid proliferation of sex cells in May. Seasonal fluctuations in glycogen content are common to all the species of oysters that have been studied. The pattern of changes varies in different localities and in different species depending on local conditions—temperature and abnormal salinity of water, abundance and type of food available, and intensity of feeding.

Seasonal changes in the glycogen content of New England oysters show a definite cycle related to gonad development and spawning. The rapid increase in the number of sex cells in the gonad exhausts the reserve materials and brings the glycogen content to its minimum, which usually occurs immediately after spawning. After a short period of relative inactivity during which the unspawned sex cells are reabsorbed the oysters begin to accumulate and store glycogen in their tissues. The process may be rapid, as for instance in September to December 1933 (fig. 357) or gradual as in the same period in 1934. The glycogen count of oysters of the same population varies from year to year. It can be seen in fig. 357 that in 1934 the content of glycogen after spawning was significantly higher than in the preceding year. Microscopic examination of these and Cape Cod oysters showed that sometimes the glycogen reserve is not depleted during the growth of the gonad and remains at a relatively high level throughout the spawning season. Another interesting fact noticeable in the annual glycogen curve is the continuing increase in glycogen during the cold months of winter when feeding ceases.

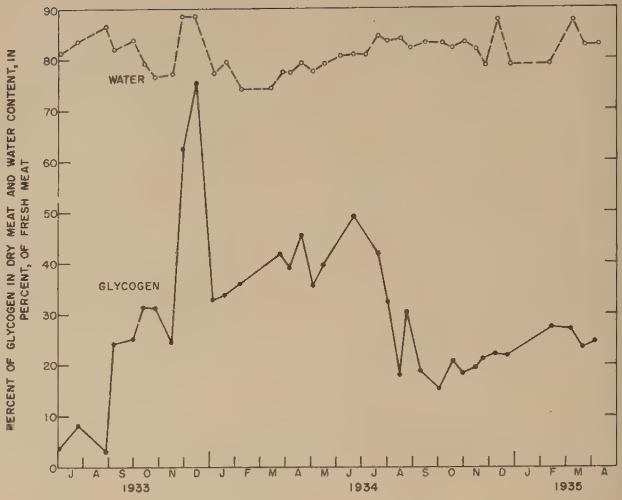

FIGURE 357.—Glycogen and water content of adult oysters (5-years-old in 1933) from commercial oyster bed off Charles Island, Long Island Sound.

The amount of glycogen stored in the tissues at a given moment is the balance resulting from the glycogen formed (glycogenesis) and that broken down (glycolysis). Biochemistry of both processes known in great detail in mammals, has not been adequately investigated in bivalves. It appears, however, reasonable to postulate that the tissue glycogen continues to be synthesized by the oyster from the carbohydrates accumulated with food during the period of active feeding or from indigenous sources of intermediary metabolism.

Increase in glycogen content is usually associated with an increase in solids and a corresponding decrease in water. There are, however, unusual instances as in the oysters found in November and December 1933 (fig. 357) which had a high glycogen content in spite of an increase of water to 88 percent and corresponding loss of solids.

The annual glycogen cycle in oysters of the York and Piankatank Rivers, Va. (Galtsoff, Chipman, Engle, and Calderwood, 1947) follows the general pattern similar to that of Long Island with the only difference that the lowest concentrations were observed in July to September and the highest in November to February. In Louisiana the period of low glycogen was found by Hopkins, Mackin, and Menzel (1954) to extend from April to the end of November. All the differences mentioned above are associated with the longer reproductive periods in warmer climates.

The cyclic change in glycogen content has been described for *O. edulis* and *C. angulata* by Bierry, Gouzon, and Magnan (1937); Bargeton (1945);

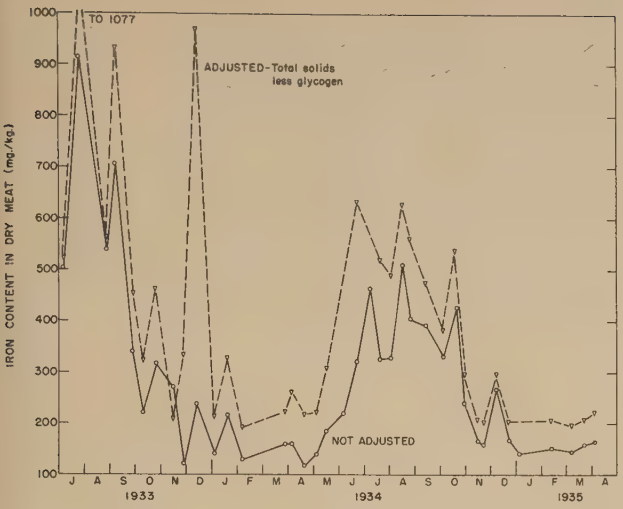

FIGURE 358.—Seasonal changes in iron content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

Gaarder and Alvsaker (1941), and many others. In general the changes are similar to those observed in *C. virginica*, the lowest content occurring during the summer.

The cycle of fat has not been studied for *C. virginica*. According to Watt and Merrill (1950) the average content of fat of raw oyster meat sold in U.S. markets is equal to 2.1 percent. Gaarder and Alvsaker (1941) found that the fat content of *O. edulis* in Norwegian ponds varied from 2.52 to 1.56 percent with the annual average of 2.17 percent. The observed fluctuations were not seasonal.

IRON, COPPER, ZINC, AND MANGANESE

The four metals present in the meat of Long Island Sound oysters were found primarily in the gills and mantle; lesser quantities were in the muscle and gonads. Only the ovaries had manganese in quantities greatly exceeding the content of this metal in other organs. These findings are based on the series of chemical analyses of different organs and on histochemical reactions used for the localization of various metals. The curves in figures 358 to 361 showing the seasonal changes in the contents of metals expressed in mg./kg. of dry weight have a common pattern despite large differences in the levels of concentration. The amounts of metals increase during summer and decline in the following fall and winter. The increase in metals during the warm feeding season cannot be associated with the possible presence of food particles in the intestinal tract, since the total weight of food and fecal masses inside the intestines constitutes only a minute fraction of the body weight, and because the mantle and gills are the principal storage places for iron, copper, and zinc. Likewise the increase in metal content is not caused by the loss of glycogen since the general trend of the curves is not affected by adjusting the values of concentrations to the weight of solids less glycogen (dotted lines in figures 358 to 361).

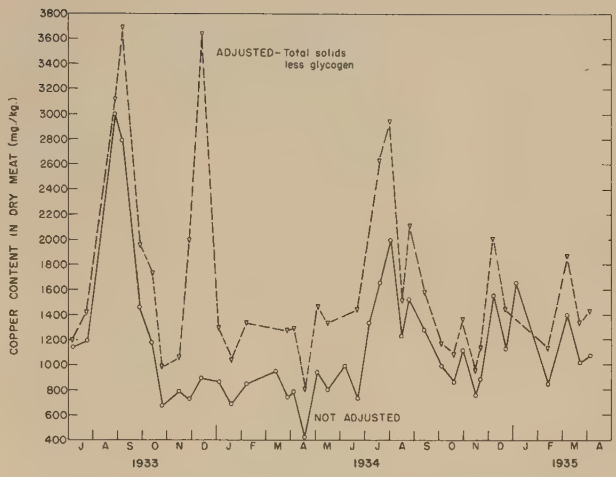

FIGURE 359.—Seasonal changes in copper content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

With minor exceptions the two types of curves (adjusted and nonadjusted) run parallel. It appears, therefore, a firmly established fact that the content of the four metals increases during the

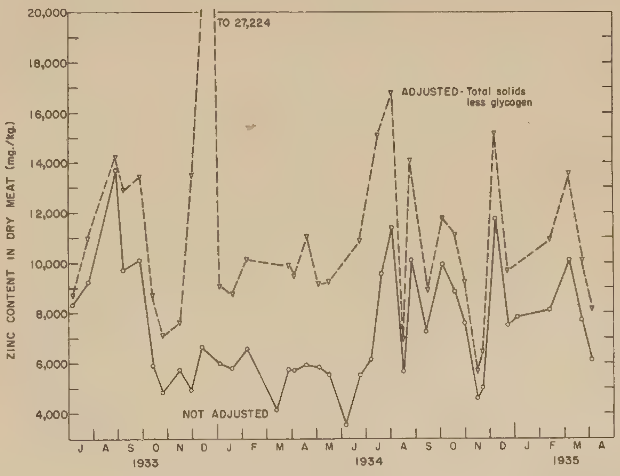

FIGURE 360.—Seasonal changes in zinc content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

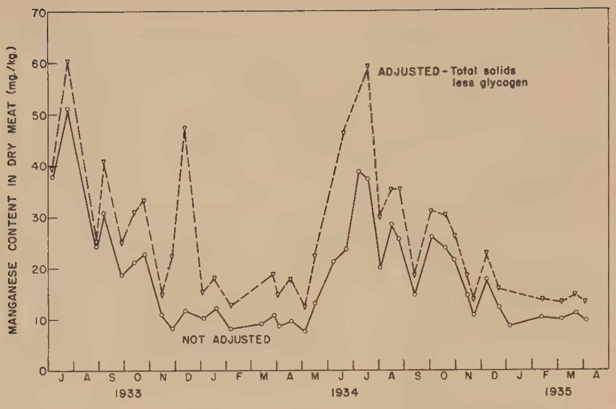

Figure 361.—Seasonal changes in manganese content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

summer and decreases in winter. Heavy metals are accumulated in the oyster tissues by direct absorption from sea water, ingestion in the intestinal tract with food, and dispersal by blood cells throughout the visceral mass.

Individual variations in iron, copper, and zinc contents are large, and oysters living side by side frequently were found to vary in the contents of these metals. This is particularly easy to observe in green oysters, for the color varies in intensity in direct relation to the copper content. In the case of pronounced green discoloration the presence of metallic copper may be demonstrated by inserting in the tissues a well-polished steel knife; the surface becomes copper plated in a short time. This simple method can be used profitably for a qualitative demonstration of the presence of copper. The green pigment of the oyster can be isolated by grinding the meats with pure sand previously treated with strong hydrochloric acid and carefully washed. The proteins in the extract are precipitated with ammonium sulfate $(NH_4)_2SO_4$, but the pigment remains in solution. It was shown by S. Lepkofsky (quoted in Galtsoff and Whipple, 1931) that the green compound is not even remotely related to hemocyanin and that it exists in the oyster as a readily diffusable material. The green extract is readily soluble in methyl alcohol, less so in ethyl alcohol, and quite insoluble in butyl or amyl alcohol. It is insoluble in chloroform, ether, acetone, or benzene, but is soluble in pyridine.

When the extract is left standing for 4 months or longer in sealed glass tubes it turns to a reddish-chocolate color, but the green color returns if it is shaken with methyl alcohol, ethyl alcohol, or pyridine. Bubbling air or oxygen fails to bring back the green color.

The content of copper in the tissues can be artificially increased by placing the mollusks in sea water containing an excess of this metal. Green discoloration develops in the oysters kept in sea water which is in contact with copper pipes or valves. Within about 6 summer weeks the copper content in oysters kept under such conditions increased up to 20 times and the meats became deep green. Analyses of samples of Woods Hole water taken in the harbor and from the laboratory supply pipe showed that the copper content in the laboratory water sometimes exceeded 20 to 40 times its concentration in the harbor near the intake pipe.

The iron content of oyster meat may be artificially augmented by adding ferric salts to the water in which the oysters are kept. The iron in sea water was enriched by suspending several pounds of iron nails in the large outdoor tank with the oysters or by adding ferrous iron sulphate (copperas). Although large quantities of iron oxide particles were formed and remained in suspension, the concentration of iron dissolved in sea water did not change significantly in 28 days but the content of iron in suspension increased about five times. Particles of iron oxide were noticeable in the feces, which contained as high as 13,000 mg. of iron per kg. (dry basis). Oysters being prepared for chemical analysis were placed for several days in running sea water containing no iron particles in suspension so that all loose sediment in the mantle cavity and the gills would be discarded. The removed meats were thoroughly inspected and rinsed in sea water. Microscopic examination of sections of the gills and other organs was made at intervals varying from 20 minutes to several days following the initial feeding with iron oxide suspension. The oysters treated with potassium ferrocyanide and hydrochloric acid (Prussian blue reaction) show that leucocytes on the surface of the gills actively ingest iron particles, migrate throughout the body, and aggregate near the wall of the intestines and in blood vessels (fig. 362). No iron was detected in the digestive diverticula, sex cells, or in the adductor muscle. Some iron is eliminated through the epithelial cells of the mantle (fig. 363).

Histological localization of copper is not entirely reliable. According to Mallory (Lillie, 1948; Glick, 1949) copper compounds produce a

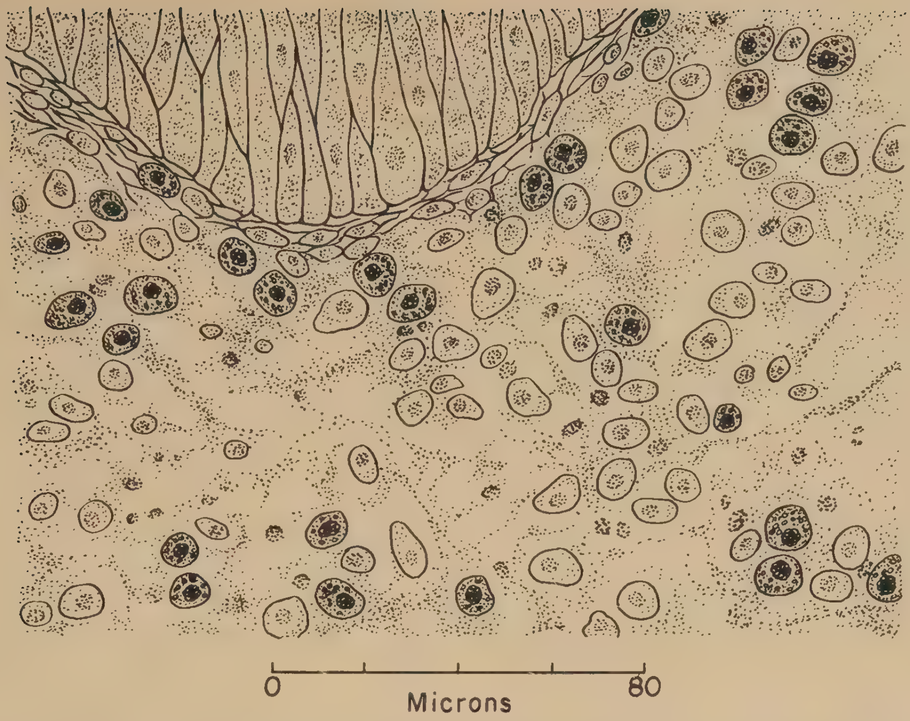

FIGURE 362.—Blood cells of *C. virginica* containing iron in the connective tissue under the digestive tract. Drawing of a section of oyster fed iron particles and treated with potassium ferrocyanide and hydrochloric acid.

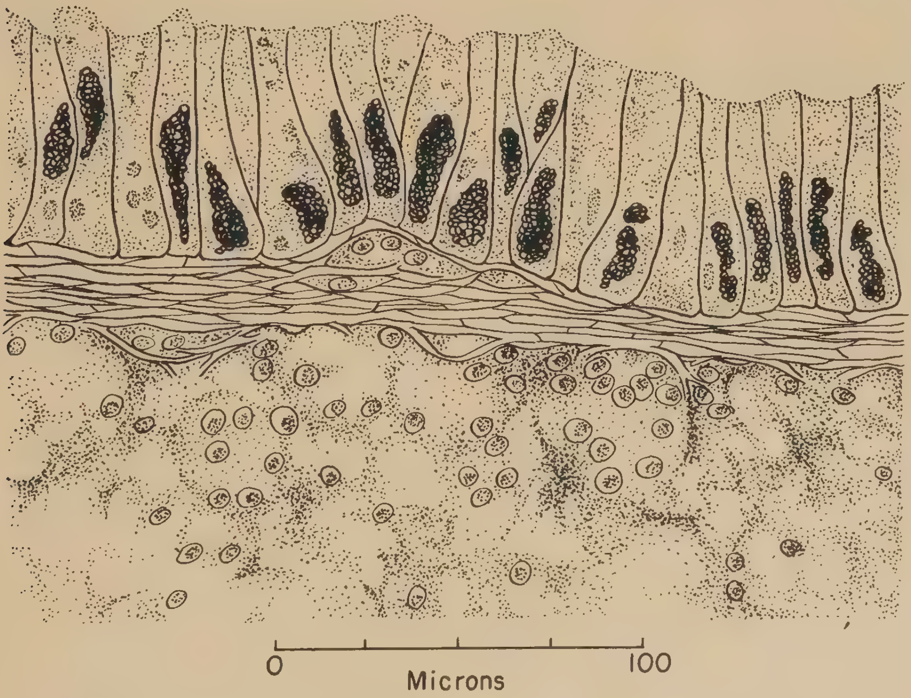

FIGURE 363.—Iron particles in the mantle epithelium of *C. virginica* fed iron oxide. Treated with ferrocyanide and hydrochloric acid.

light to dark blue color with an unoxidized fresh aqueous solution of hematoxylin made by dissolving from 5 to 10 mg. of pure hematoxylin in 0.5 to 1 ml. of 100 percent ethyl alcohol and 10 ml. of distilled water boiled 5 minutes to drive off carbon dioxide. Sections of celloidin-embedded tissues were stained for 1 hour or longer. Copper compounds appeared as a light to clear blue color. The reaction is to a certain extent obscured by a mass of yellow to brown colors produced by the iron in the tissues. The surface of the mantle and gills of green oysters usually contains large masses of blood cells loaded with dark granules which react strongly with Mallory reagent. It is obvious that a large proportion of the copper in the oyster is found in the blood cells.

For histological localization of zinc, the nitroprusside reaction proposed by Mendel and Bradley (1905) can be used. The reaction is considered by Lison as specific (Lillie, 1948). The method involves treatment of the paraffin section of tissues for 15 minutes at 50° C. in 10 percent sodium nitroprusside solution. The section is washed for 15 minutes in gently running water. Then a drop of sodium or potassium sulfide solution is introduced under one side of the cover glass. The reagent elicits an intense purple color in the zinc precipitated by the nitroprusside. In many preparations of green oysters treated by this method a diffuse purple coloration of varying degrees of intensity was produced in different organs, the mantle and gills staining conspicuously deeper than the rest of the body. The concentration of zinc within the blood cells could not be demonstrated by this method. It appears probable that zinc is present in a soluble state and is more universally distributed through the tissues than iron, copper, and manganese. Observations on the uptake and accumulation of radioactive zinc Zn^{65} confirm this view. Chipman, Rice, and Price (1958) demonstrated that zinc in surrounding water is rapidly taken up in great amounts by the bodies of oysters, clams, and scallops. The gills of oysters were found to accumulate almost twice the concentration of radioactive zinc, as did the organs and tissues. The digestive diverticula and body mass contained a considerable amount of Zn^{65}. The zinc content of sea water along the Atlantic and Gulf of Mexico inshore waters averages 10.6 μg./1.

Several histochemical reactions for the localization of manganese in the oyster tissue have been tried without success. So far as I know there is no satisfactory method for demonstrating this element in the cells and tissues.

The distribution of manganese in the oyster body is related to the female reproductive cycle, because the concentration of this element in fully developed ovaries (see fourth column of table 42), is 15 times that of the spermary (Galtsoff, 1943) and its total concentration materially decreases after the discharge of eggs. No such relationship is apparent for the other three metals.

The role of heavy metals in the physiology of the oyster is not clear. It is reasonable to assume that manganese performs some function during the rapid propagation of ovocytes, possibly as a catalyst.

Iron, copper, and zinc may be stored in the tissues and in some blood cells as excess materials which are slowly eliminated. Observations on excretion of iron by the mantle epithelium (fig. 363) and accumulation of iron, copper, and zinc in the mantle and gills support this view. The distribution of the four metals in different organs of Woods Hole oysters was studied analytically. The organs were excised by fine scissors, weighed, and analyzed separately. The results of the analyses are shown in table 42 as means of 10 samples taken from natural environment. The lower part of the table summarizes the results obtained after keeping the oysters in a tank with an excess of copperas. It appears significant that both mantle and gills have absorbed relatively large quantities of the metals.

TABLE 42.—*Distribution of metals in the body of adult* C. virginica *in Cape Cod waters (mg./kg., dry weight)*

[Mean of 10 samples. Early August and October, 1936]

Body portion*	Iron	Copper	Zinc	Manganese	Remarks
Gills	382	178	4,480	39	Summer samples from natural environment.
Muscle	136	65	1,420	4	
Ovary	151	63	1,710	60	
Spermary	136	65	1,420	4	
Residue	252	153	4,630	9	
Mantle	184	1,840	22,000	14	Autumn samples from tanks with excess of copperas after 26 days of exposure.
Gills	194	1,920	19,400	25	
Muscle	75	172	1,590	4	
Residue	401	1,490	14,400	9	

*In summer the mantle could not be separated without contaminating the sample with underlying gonads; in the autumn, after spawning, the gonads contain only few undifferentiated cells of germinal epithelium.

VARIATIONS IN THE CONTENT OF PROTEINS, AMINO ACIDS, AND VITAMINS

The protein content in oyster meat of *C. virginica*, determined by the Kjeldahl method as N × 6.25, fluctuates between 5.1 and 9.8 percent of a fresh wet sample and between 42 and 57 percent of a dry sample. The figures quoted above from the paper by Wentworth and Lewis (1958) refer to the oysters of Apalachicola Bay, Fla., the extent of fluctuations is probably common to all oysters of the Atlantic and Gulf states since occasional observations on oysters from various states fall within this range (Jones, 1926). According to monthly observations by Gaarder and Alvsaker (1941), the protein content in the meat of *O. edulis* from Norwegian ponds ranks somewhat higher, varying from 8.8 to 11.2 percent (fresh, wet basis) with an annual average of 10.5 percent.

Interesting biological observations were made by Duchâteau, Sarlet, Camien, and Florkin (1952) on free amino acids in the muscles of marine bivalves, *O. edulis* and *Mytilus edulis*, and the fresh-water mussel, *Anodonta cygnea*. The muscles of these mollusks were isolated after bleeding, boiled for 5 minutes to inactivate proteolytic enzymes, homogenized, and treated with tungstic acid. Protein-free samples were hydrolyzed and analyzed. The results (table 43) show that the amino acid contents differ greatly between the marine and fresh-water species. Generally higher concentrations of amino acids in the muscles of marine forms is related to the osmotic equilibrium with the blood, which in these animals has nearly the same concentration as that of sea water. Because the concentration of inorganic ions in the tissues is lower than in the blood, a relatively high concentration of free amino acids in the tissues is necessary for maintaining osmotic equilibrium.

The concentration of protein in blood plasma in *O. edulis*, *Pecten maximus*, *Mya arenaria*, and *Mytilus edulis* is about 0.1 percent (Florkin and Blum, 1934). Samples of blood were collected after tearing off the gills, and the cells were removed by centrifugation.

Table 43.—*Free amino acids (mg./100 g. of water) in the muscles of marine and fresh-water bivalves**

	Ostrea edulis	*Mytilus edulis*	*Anodonta cygnea*
Alanine	646. 0	340	8. 8
Arginine	66. 6	415. 5	36. 5
Aspartic acid	26. 1	200. 4	4. 4
Glutamic acid	264. 0	317. 0	29. 4
Glycine	248. 0	399. 0	13. 2
Histidine	22. 9	12. 1	2. 5
Isoleucine	19. 2	24. 8	6. 3
Leucine	12. 9	15. 4	3. 6
Lysine	22. 0	39. 4	8. 2
Methionine	8. 4	9. 8	0. 4
Phenylalanine	8. 5	9. 6	1. 6
Proline	166. 0	29. 0	1. 0
Threonine	9. 7	30. 5	3. 6
Tyrosine	10. 3	12. 7	2. 2
Valine	10. 8	14. 4	3. 3

*From: Duchâteau, Sarlet, Camien, and Florkin, 1952. The French investigators express the concentration in a rather unique manner as mg./100 g. of "d'eau de fibre."

Nutritional studies have been made by feeding raw and frozen oysters to albino rats suffering from artificially induced vitamin deficiency (Randoin and Portier, 1923; Jones, Murphy, and Nelson, 1928; Whipple, 1935). Experimental results showed that oysters are a good source of vitamins A, B, and D. Daily feeding of 2 g. of fresh Chesapeake Bay oysters (0.32 g. on a dry basis) furnished sufficient vitamin A to cure rats of xerophthalmia (chronic inflammation and thickening of the conjunctiva of the eye) in 18 to 20 days. According to Whipple's data the vitamin content of oysters taken in October from Great South Bay, Long Island, N.Y., was approximately three U.S.P. units/g. The vitamin D content of oysters harvested from the same bay in the fall was approximately 0.05 U.S.P. units/g. and the vitamin B (B^1) content was found by Whipple to be approximately 1.5 Sherman units/g. Oysters are a very modest source of vitamin D and their antiricketic value is low.

In more recent work Wentworth and Lewis (1958) determined by chemical analyses the contents of niacin, riboflavin, and thiamine (table 44). None of these vitamins was found to have a distinct pattern of seasonal fluctuation.

Table 44.—*Range of vitamin contents in Apalachicola, Fla., oysters in mg./100 g. wet weight*

[From February 1955 to August 1956 From Wentworth and Lewis, 1958]

	February	August
Niacin	1. 41	2. 52
Riboflavin	0. 06	0. 28
Thiamine	0. 08	0. 13

Thiamine content of raw shucked oysters studied by Goldbeck (1947) varied by region. Oysters collected in the waters of Connecticut and New York contained more thiamine per unit of fresh weight than those from Louisiana, Georgia, Virginia, and Maryland (table 45). The determination of thiamine was made by chemical method (using thiochrome) and by rat growth method, which gave values about 9 percent smaller than the chemical tests.

The sterol mixtures of bivalves are of particular interest, because in certain species they are the richest natural sources of provitamin D. In *Modiolus demissus* of the Atlantic coast of America the content of provitamin D was found to be sufficiently rich to warrant commercial exploitation for the manufacture of vitamin D preparations (Bergmann, 1962).

The cholesterol in bivalves constitutes but a small portion of the sterol mixtures in comparison with those obtainable from gastropods. In *C. virginica* and *C. gigas* Bergmann (1934) found a new sterol which he named ostreasterol. Similar compounds found in the sponge *Chalina* and in Japanese oyster (*C. gigas*) which were named chalinasterol and conchasterol. Reinvestigation of bivalve sterols proved the identity of ostreasterol, chalinasterol, and conchasterol with 24-methylenecholasterol (Bergmann, 1962).

The conditions and type of food which favor the enrichment of the bivalve body with sterols and vitamins are not known.

Table 45.—*Thiamine contents per 100 g. of raw oysters from different states*

[From Goldbeck, 1947]

State	Thiamine in µg.	State	Thiamine in µg.
Connecticut	170	Virginia	100–110
New York	170–180	Georgia	98–106
Maryland	100–103	Louisiana	110–130

CONDITION INDEX

Oysters of good quality have relatively large amounts of meat in relation to their total volume. Their glycogen content is high, and the meat has a creamy color and pleasant flavor. Determination of glycogen and of the total solids is a time-consuming procedure which cannot be regularly used in the oyster trade. To Caswell Grave (1912) belongs the credit of expressing the quality or fatness of oysters as the percentage of the volume of space enclosed between the two valves occupied by the oyster body. Hopkins (quoted from Higgins, 1938, p. 49–50) developed this idea further and suggested that the ratio

$$\frac{\text{dry weight of meat in g.} \times 100}{\text{volume of cavity in ml.}}$$

is a useful index of quality. Since then the ratio between the dry or wet weight of meat to the volume of the cavity has been used by many investigators in determining the condition index of oysters. The volume of the cavity can be measured by displacement. The oyster shells are thoroughly scrubbed with a wire brush, and each oyster is placed in a glass container provided with a side arm set at an angle to the side wall of the container (fig. 364). First the zero level of water is marked; then the oyster is introduced, and the level is brought back to zero position by draining the water through a drain pipe at the bottom. The water is collected, and its volume measured. The oysters are then taken from the container, opened carefully, and the meats removed. The volume of shells without meat is measured, and the volume of shell cavity is found by the difference between the volume of the whole oyster and the volume of its shell.

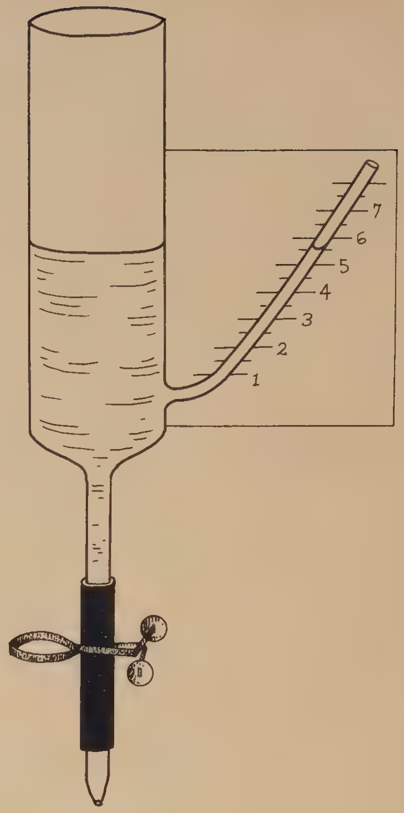

Figure 364.—Glass container used for determining the volumes of whole oysters and oyster shells by displacement.

The methods used in determining condition index have not been standardized and, therefore, the values given by different investigators vary. Baird (1958) applied statistical analysis in evaluating the significance of variation of the index. He also demonstrated that little accuracy is gained by using dry weights as an index measurement and that even with fairly large samples the fluctuations may be considerable. He concludes that 50 oysters per sample is the largest practicable number.

The condition index has a practical use for oyster growers. It gives an objective method of comparing the quality of oysters taken from different commercially exploited oyster beds, but this comparison is valid only if oysters of the same species and of approximately the same age are used. Oysters from overcrowded natural reefs and young oysters are usually flat, with very little inner space between the valves; consequently their condition factor will be relatively high because the bodies occupy almost the entire shell cavity.

Westley (1961) found that the condition index of samples of *C. gigas* in Oakland Bay, Wash., in 1956 varied between 6.2 and 8.1. The condition index of the oysters in North Bay, Wash., in August 1957 was 12.3 to 15.0.

Observations were made on *O. lurida* in Oyster Bay on July 11 and repeated on August 8, 1957. On the first date the range of condition index varied from 6.6 to 7.2, while a month later it had increased to 16.0 to 17.3. The improvement was probably associated with an accumulation of glycogen after discharge of the larvae. In this case the volume of the oysters was measured by weighing them first in air and then in water, and computing the volume from the difference between the weights. The removed meats were oven-dried to constant weight (at 100° C.).

Although the condition index may be useful to oyster growers as a measure of quality of oyster meats, it provides no advantages for physiological studies, and cannot be used for the study of growth.

ANTIBACTERIAL AGENTS

Antibacterial and antiviral agents were found in the meat of the oyster (*C. virginica*), in abalone (*Haliotis rufescens*), and in a number of other mollusks. These substances have been isolated in the laboratory of the U.S. Public Health Service and their activity tested in vitro on a number of pathogenic bacteria (Li, Prescott, Jahnes, Martino, 1962), and on various strains of influenza and polio viruses. In vitro tests were made by using cultures of monkey kidney tissue; tests in vivo were conducted by feeding white mice with the extracts and recording the death rate after infection. There are two different extracts which the authors call Paolin 1 and Paolin 2 (according to Li, Paolin is a Chinese word which means "abalone extract"). Paolin 1 was found to inhibit the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhosa*, *Shizella dysenteriae*, and others. Paolin 2 fed to white mice decreased the death rate of animals experimentally infected by virus. The decrease was from 36 percent in the controls, which received no extracts, to 10 percent in the animals fed with oyster or abalone extracts before infection (Li and Prescott, 1963). The discovery by Li and his coworkers is of great practical significance and opens a new chapter of research into the role of antibacterial and antiviral agents in the tissues of mollusks.

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CHAPTER XVIII

ENVIRONMENTAL FACTORS AFFECTING OYSTER POPULATIONS

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The various species of the family Ostreidae inhabit the coastal waters within the broad belt of the sea, limited by the latitudes 64° N. and 44° S. Some large aggregations cover many square miles of bottom of littoral and intertidal zones; they also thrive above the bottom attached to rocks and underwater structures, branches and trunks of fallen trees, and miscellaneous objects. These aggregations of live oysters and empty shells are called oyster bottoms, oyster beds, oyster banks, or oyster reefs. The expressions are not well defined either biologically or in the legal sense and are used interchangeably. Only those species of oysters which form large and dense populations are important to man as a source of food. Those living singly and widely dispersed are of no commercial value.

Descriptions of oyster bottoms found in the world literature combined with personal observations over the course of years in the United States, France, Italy, the West Indies, Cuba, Venezuela, Panama, Hawaii, and some of the South Pacific islands have convinced me that, regardless of the species of oysters present, certain major factors are common to all oyster bottoms.

It is a matter of historical interest that more than 80 years ago Möbius (1883) established the concept of a biocenosis or a social community using an oyster bank as an example. According to his definition every oyster bed is to a certain degree—

> . . . a community of living beings, a collection of species, and a massing of individuals, which find here everything necessary for their growth and continuance, such as suitable soil, sufficient food, the requisite percentage of salt, and a temperature favorable to their development. Each species which lives here is represented by the greatest number of individuals which can grow to maturity subject to the conditions which surround them, for among all species the number of individuals which arrive at maturity at each breeding period is much smaller than the number of germs produced at that time. The total number of mature individuals of all the species living together in any region is the sum of the survivors of all the germs which have been produced at all past breeding or brood periods; and this sum of matured germs represents a certain quantum of life which enters into a certain number of individuals, and which, as does all life, gains permanence by means of transmission.

Möbius further commented that a change in one factor of a biocenosis affects other factors of the environment and eventually changes the community character. Relative abundance of various species constituting a bottom community is affected by changes in estuarine environment and by man's activities which alter the environment. Patterns of currents, salinity gradients, and turbidity of water may be changed by dredging operations, construction of inshore installations, and other harbor and waterway improvements. Commercial dredging removes substantial portions of natural oyster grounds; planting of seed oysters for growing increases artificially the population densities. Inadvertent introduction of foreign species, competitors, and predators disturbs the established biological balance. Finally, excessive discharge of domestic sewage and trade wastes causes irreparable damage to productive oyster bottoms.

The productivity of a sea bottom may be measured by determining the sum of weights of all animals and plants in a unit of area. The value, called community biomass, is of considerable theoretical interest to the marine ecologist engaged in the study of oceanic productivity. It has, however, no practical application in determinations of the productive capabilities of a community dominated by a single species such as oyster, clam, or scallop. The species productivity of any bottom may be materially reduced by competitors, predators, and other conditions that may suppress the reproduction and growth of commercially utilizable organisms while not affecting or even sometimes encouraging the growth of noncommercial forms.

Descriptions of oyster bottoms usually provide information regarding their location, type of bottom, depth and salinity of water, the principal species associated with oysters, and the abundance or absence of predators. This type of description is found in the papers of Dean (1892) on South Carolina grounds; Moore on the condition and extent of oyster grounds in Texas (1907), Louisiana (1899), Mississippi Sound (1913), James River, Virginia (1910), Delaware Bay (1911); Pearse and Wharton (1938) on oysters of Apalachicola Bay; Frey (1946) on oyster bars in the Potomac River; Hagmeier and Kändler (1927) on oyster banks in North Freisland shoals, Germany; Joubin on the coast of France (1906, 1908), and many others.

Because of the great diversity in the kind and number of species forming an oyster community only a few generalizations can be drawn from descriptive data: 1) in common with other bottom communities, oyster grounds of the warm southern waters support a greater variety of species than do the colder waters of the northern latitudes, and 2) the variety of plant and animal species is less in waters of low salinity than in adjacent areas of higher salt concentrations.

The inferences are in accord with observations made by European ecologists and summarized by Hedgpeth (1953). In the Elbe estuary the weight of all invertebrates per square meter of bottom decreases from 6,068 g. in the area of full oceanic salinity to only 37 g. in brackish water. A similar decrease in the weight of community biomass is found along the northern coast of Germany, although the difference is much smaller ranging from 304 g. per square meter of sea bottom to 16 g. in the inshore areas. The decrease in the biomass cannot be attributed to a single factor of the environment since other conditions such as rate of water movements, sedimentation, and food content are associated with the salinity changes.

There are many well-documented cases of destruction of productive oyster bottoms by human activities. Möbius (1883) cites formerly rich oyster beds of Cancale, Rochefort, Marennes, and Oléron on the West Coast of Europe in which the oyster populations were replaced by cockles and mussels. The newcomers were present in small numbers while the oysters flourished but greatly increased in abundance when the removal of oysters left more space for them to settle.

Many well-documented examples may be cited of the destruction of oyster bottoms by sand and mud stirred up by dredging operations in nearby areas. One incidence of this nature occurred in 1935 to 1938 near the Buzzards Bay entrance to the Cape Cod canal, Mass., where valuable oyster grounds were buried under 8 to 12 inches of material that was disturbed by dredging and then settled on the oyster grounds. Three to four years later the area was repopulated by quahogs and continues to remain highly productive, although the species composition has been completely changed.

Discounting minor local variations, the basic requirements of the oyster are identical regardless of the location of the oyster bottoms. The suitability of a bottom area for the development of a productive oyster community can, therefore, be evaluated if the effects of different environmental factors are estimated.

Principal factors favorable for the propagation, growth, and general welfare of an oyster community are character of bottom, water movements, salinity of water, temperature, and food. The unfavorable or destructive factors that tend to inhibit the growth and productivity of a community are sedimentation, pollution, competition, disease, and predation. The interaction of these five positive and five negative factors acting simultaneously on a community determines its utilizable productivity.

POSITIVE FACTORS OF ENVIRONMENT

CHARACTER OF BOTTOM

Oysters may grow equally well on a hard, rocky bottom or on semihard mud firm enough to support their weight. Shifting sand and soft mud are the only types of bottoms which are totally unsuitable for oyster communities. With the exception of these extreme conditions oysters adapt themselves to a great variety of bottoms. They thrive well on shore rocks and underwater structures which are left exposed at low tide. The controlling factor in this situation is the climate, since no oyster can survive several hours exposure to below freezing temperature, and, therefore, none are found growing near the surface in the latitudes where in winter they may be killed at low tide, or frozen in the ice and carried away by tidal currents. The degree of softness and instability of the bottom can be quantitatively measured by penetrometers, and by determining the amount of bottom material transported by currents of different velocities. The depth of sinking into a mud layer of a probe of known dimensions under constant weight can be used as a measure of the relative softness of sediment. The determination must be made in situ because the removal of a sample and handling in the laboratory changes the consistency of mud. A penetrometer to evaluate certain physical properties of marine sediments in situ has been designed by Miller (1961). The instrument is a conical probe which is driven at low, constant speed into the bottom; resistance to penetration as the probe sinks into the bottom is recorded graphically. An instrument answering these specifications and constructed at the University of Rhode Island has been used in research at the Narragansett Marine Laboratory at Kingston, R.I., and has proved reliable in providing information about compactness, degree of plasticity, allowable bearing loads, and other properties of sediments. No reliable method has yet been developed for measuring the resistance of sediment to water current in the sea.

A soft muddy bottom may be artificially improved by planting oyster or clam shells to attain the desired firmness. Other materials, such as gravel and slag from blast iron furnaces, have been tried experimentally but are less satisfactory, primarily because of the greater weight and higher cost. At present the reinforcement of oyster bottoms by shells remains the principal practical method used on a large scale for the improvement of oyster bottoms or for the establishment of new ones.

Soft muddy bottoms may be gradually converted by the oysters themselves into oyster banks or reefs because of an innate ability of larvae to choose a substratum upon which to settle. This ability is probably common to most species of bottom invertebrates having free-swimming larvae (Verwey, 1949). The process begins with the attachment of several larvae to a single shell or other hard object lying on the surface of the mud. Other larvae attach to those that have already settled, and soon a cluster of oysters is formed on the surface of the mud (fig. 365).

Dead oyster shells dropping from clusters provide additional surfaces, and the reef begins to grow horizontally and vertically. The process is typical for the tidal flats of South Carolina and Georgia where successive phases can be easily observed. Oysters grown on mud have long, slender shells.

The suitability of bottom to an oyster community may be expressed by an arbitrary scale from 0 to 10, according to the relative softness and stability. Bottom conditions fully unsuitable for the formation of any oyster community may be designated as zero. The zero value of any positive factor denotes conditions under which the community cannot exist, regardless of the values of all other factors of the environment. The zero value of bottom factor refers either to extremely soft mud not capable of supporting the weight of an empty shell or to shifting sand; both conditions are unsuitable for oysters. Marginal conditions are indicated by 1, and optimum conditions by 10. The highest value of bottom factor may be assigned to firm and stable bottoms such as rocks and hard or sticky mud. The value of 1 is assigned to the soft muddy bottoms of the South Atlantic States and Texas.

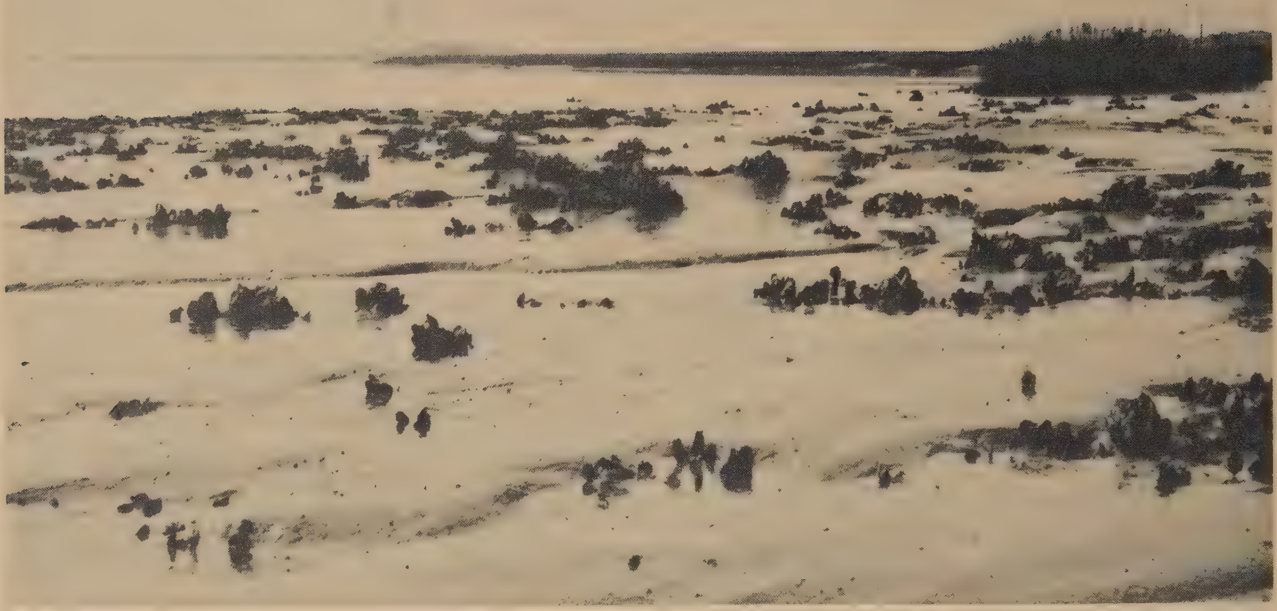

Figure 365.—Initial stage in the formation of an oyster bank on very soft mud of a tidal flat. Photographed at low tide near Brunswick, Ga.

WATER MOVEMENTS

Free exchange of water is essential for the growth, fattening, and reproduction of oysters. An ideal condition is represented by a steady, nonturbulent flow of water over an oyster bed, strong enough to carry away the liquid and gaseous metabolites and feces and to provide oxygen and food. Furthermore, an oyster bed can expand only if the larvae are carried by the currents and at the time of setting are brought in contact with clean, hard surfaces. Estuaries seem particularly suitable for the expansion of oyster communities and for the annual rehabilitation of oyster populations reduced by harvesting because some larvae, carried back and forth by the oscillating movements of tidal waters, eventually settle beyond the place of their origin.

In large embayments, such as Long Island Sound, the difference between the surface and bottom salinities is small, about 1°/₀₀ or 2°/₀₀. In tidal rivers and true estuaries the differences between the salinities of the lower strata and those at the surface are considerable. Salinity stratification, as will be shown later, complicates the pattern of circulation.

The great variety of conditions found in the bodies of water within the tidal zone makes it difficult to define the term "estuary" in a few precise words. A Latin dictionary (Andrews, 1907) defines the word "Aestuarium" or "Aestus" as a part of the seacoast overflowed at flood tide but at ebb tide left covered with mud and slime. Some authors extend the concept of an estuary to include such large bodies of water as the Mediterranean Sea and the Gulf of Mexico, while others restrict the use of the term to relatively small coastal indentures in which the hydrographic regime is influenced by the river discharge at the head and the intrusion of sea water at the mouth. Cameron and Pritchard (1963) define an estuary as "a semienclosed coastal body of water having free connection with the open sea and within which the sea water is measurably diluted with fresh water deriving from land drainage." The essential features of a true estuary are the inflow of river water at the head and the periodical intrusion of sea water at its mouth. Stommel (1951) classifies estuaries by the predominant causes of movement and mixing of water, which may be due either to tide, wind, or river flow. Rochford (1951) points out the significant differences between brackish water and estuarine environment. According to his ideas, brackish water refers to a dynamically stable environment of lakes, lagoons, etc., in which sea water, diluted by fresh water, is not necessarily influenced by tidal movements. On the other hand, the estuarine environment, influenced by tidal rise and fall, is dynamically unstable.

The persisting factors of a typical estuarine environment are seasonal salinity variations and circulation exchange between the river and sea water. The intruding salt water forms a wedge or prism with its base at the mouth and the tip at the upper part of the estuary. The position of the wedge along the bottom and its dimensions depend to a great extent on the flow of the river water.

Circulation and mixing of water is a highly complex problem adequately discussed in the papers of Rochford (1951), Ketchum (1951a, 1951b), Pritchard (1951, 1952a, 1952b), and in the textbook, The Sea, vol. II, edited by Hill (1963).

It is important for a biologist to understand that the type of circulation that prevails in a specific estuary depends on physical structure, i.e., size, depth, bottom configuration, etc., river flow, and vertical salinity gradients along the entire length from head to mouth. Circulation pattern and mixing have important biological implications in the study of the distribution and transport of sediments, pollutants, and plankton, including free-swimming larvae of sedentary invertebrates.

The volume of fresh water entering at the head of an estuary occupies the upper layer and exceeds the volume of the up-estuary flow in the lower and more saline layer by an amount sufficient to move the fresh water toward the sea (Pritchard, 1951). As one moves seaward, the volume of saline water contributed by the ocean increases while the river water, entered at the head of the estuary is being removed. The process of removal of river water, called flushing, continues throughout the estuary from its mouth to the so-called "inner end" which is defined by Ketchum (1951b) as "the section (of an estuary) above which the volume required to raise the level of the water from low to high water mark is equal to the volume contributed by the river during a tidal cycle." Consequently, there will be no net exchange of water through this section during a flood tide and the water above the section should be completely fresh.

The salinity at any location below the inner end varies with tide but returns to substantially the same level on successive tidal stages. Assuming that a net seaward transport of fresh water during any tidal cycle is equal to the volume introduced by the river in the same period of time, and that there is no net exchange of salt water through the cross section during the same tidal cycle, Ketchum (1951b, 1954) advanced a simplified theory which permits an easy calculation of the proportion of water removed by the ebb tide. This theory is based on the assumption that in each of the volume segments, limited by the average excursion of water on the flood tide, the water is completely mixed at high tide. Accepting this assumption, which is obviously a simplification of the actual conditions, the rate of exchange in a given segment (r_n) has the value $r_n = \frac{Pn}{Pn+Vn}$ in which Pn is the intertidal volume and Vn the low tide volume of the segment n. The average length of time required for the river water with a particle suspended in it to move through a segment of an estuary is called flushing time, which is defined as a ratio obtained by dividing the volume of river water, calculated from the salinity data, by river flow. The ratio is expressed in number of tides. In Raritan Bay, N.J., a survey made by the Woods Hole Oceanographic Institution indicates the flushing time for the entire estuary was 60 tides.

If a stable pollutant is discharged at a constant rate at the head of an estuary and is uniformly mixed as it is transported downstream, its proportion in the water can be calculated by using the formula of Hotelling which was applied in determining the concentration of pollutant over Olympia oyster beds (Hopkins, Galtsoff, and McMillin, 1931). According to this formula the proportion p of a contaminant in a basin is:

$$p = \frac{a}{a+b}\left[\frac{1^{-}(1-a+b)^{t}}{V}\right]$$

where a is the rate of discharge of contaminant in acre-feet per day, b the rate of influx of water into the basin in acre-feet per day, V the total volume of the basin in acre feet, and t time in days since the pollution started.

In this formula it is assumed that the influx and efflux occur discontinuously once a day. By assuming that the influx of contaminated water and the efflux are continuous, Tuckerman (see: Galtsoff, Chipman, Engle, and Calderwood, 1947, p. 100) arrives at the following formula:

$$p=p\infty[1-(1-K)^t]$$

where $p\infty$ is the limit which the proportion of contaminated water approaches after a long time and $K=\frac{a+b}{V}$. Computations made by using the two formulas indicate the same value for the $p\infty=\frac{a}{a+b}$, but differ in the rate at which this limit is approached. For the small values of $K=\frac{a+b}{V}$ which are usually encountered in estuaries, the two rates are practically identical and the simpler Hotteling formula may be applicable.

In many estuaries the water is stratified. With relation to stratification and circulation patterns Pritchard (1955) distinguishes four types of estuaries—highly stratified (type A), moderately stratified (type B), and virtually homogeneous (types C or D). The reader interested in the dynamics and flushing in different types of estuaries should consult the original contributions of Pritchard (1952a, 1952b, 1955) and Pritchard and Kent (1953) in which the complex hydrodynamical problem is analyzed. It is sufficient for a student of oyster ecology to realize that vertical and horizontal distributions of oyster larvae will be different in each of the four principal types of estuaries.

A free-swimming organism such as bivalve larva cannot be considered in the same manner as a material dissolved in water or as an inanimate body passively transported by water movements. Larvae of bivalves, barnacles, and other invertebrates may have a tendency to swarm and, therefore, their distribution may not be uniform even in a homogeneous environment. Oyster larvae react to changes in the environment by periodically closing their valves and dropping to the bottom or by swimming actively upward or in a horizontal plane. Consequently, they may be carried upstream or downstream according to their position in the water column. Field observations in the estuaries of New Jersey and Chesapeake Bay (Carriker, 1951; Manning and Whaley, 1955; Nelson, 1952) in which the salinity of water increases from surface to bottom indicate that vertical distribution of larvae is not uniform. The late umbo larvae of *C. virginica* have a tendency to remain within the lower and more saline strata, and are probably stimulated to swim by the change in salinity at flood tide. A brood of larvae swimming within a given salinity layer will be passively moved in the direction of the current. Nelson observed that in Barnegat Bay, N.J., the brood of larvae of setting size was carried about 3 miles up the bay in a single evening spring tide. In Yaquina River, a small tidal stream along the ocean shore of Oregon, swimming larvae were transferred by tidal currents and set several miles above the natural beds (Fasten, 1931; Dimick, Egland, and Long, 1941). There are many other places where setting grounds are far above the spawning grounds. Since the seaward discharge of water in an estuary usually exceeds the landward movement, it was difficult to visualize a mechanism by which the larvae can be transported up an estuary and left there. The existence of such a mechanism became apparent, however, from the hydrographic studies by Pritchard (1951). He found that estuaries may be considered as being composed of two distinct layers: an upper layer in which the net movement is toward the mouth (positive movement), and the lower layer in which the net movement is toward the head of the estuary (negative movement). There is a boundary between the two layers which may be called "the level of no net motion" (fig. 366). Since the net movement seaward does not result in a net displacement of the lines of constant salinity in the upper layer, there must be a progressive transfer of the deeper, higher salinity water of the lower layer upward, across the boundary level, to be included in the seaward transfer. The role of the strongest tidal currents is primarily that of providing energy for the mixing processes. Computations made by Pritchard show that superimposed on tidal oscillation there is a residual or nontidal seaward drift on the surface and a net landward drift along the bottom. He applied his theory to a study of the seed oyster area of the James River, Va., and found that below a depth of about 10 feet there is a net (or residual) upstream movement of water at an average speed of slightly more than one-tenth of a knot. This is the type of estuary

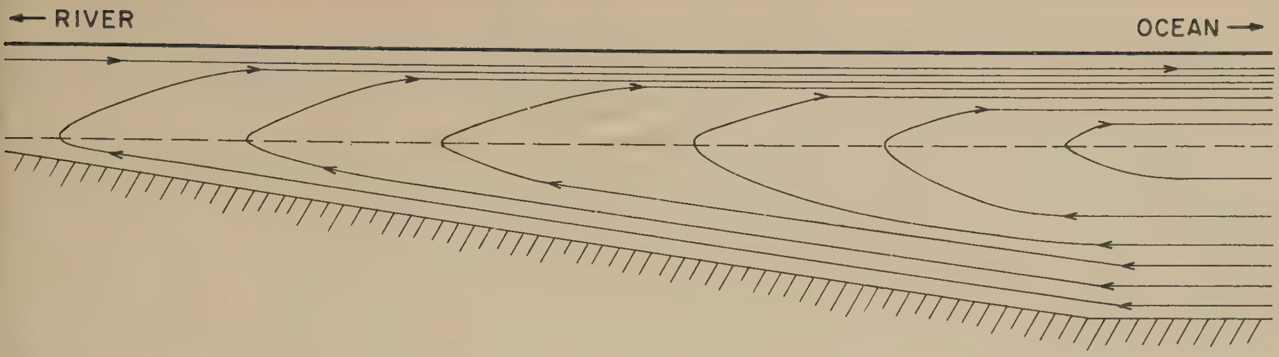

FIGURE 366.—Estuarine structure and net (i.e., nontidal or residual motion of water). Dotted line marks the boundary between the upper and lower net movement; the boundary is the level of "no net motion." From Pritchard, 1951, fig. 3.

in which larvae move from the spawning grounds at the mouth of the river up into the seed oyster bed area. Bousfield (1955) applied Pritchard's ideas to an analysis of distribution of barnacle larvae in the Miramichi estuary, New Brunswick, Canada. The retention of larval populations in this estuary was found to be due to a combination of two factors: changes in the average vertical distribution of successive larval stages, and the strength and direction of transport by residual drift at different depths. This theory of larval retention and the mechanism of transport is not applicable to bodies of water in which there is no salinity stratification or where the residual upriver drift is insignificant.

Least favorable in the life of an oyster community are occasional turbulent currents of high velocities which may dislodge and carry away young and even adult oysters not attached to the bottom. Oysters attached to rocks and other structures are not destroyed by strong currents, but their valves are injured by small pebbles and sand acting as an abrasive material. Live oysters with shells damaged by abrasion can be found in the Sheepscot and other tidal rivers along the coast of Maine.

Continuous renewal of sea water running over a bottom in a nonturbulent flow is the most desirable condition for a flourishing oyster community. On the basis of experimental studies discussed in chapter IX, p. 195, it may be assumed that under optimal conditions of temperature and salinity an average adult *C. virginica* transports water at the rate of 15 l. per hour. With 250 large oysters to a bushel and 1,000 bushels per acre, an oyster bed of that size would require 3.75 million liters of water per hour. My observations show that under the best of conditions oysters can take in water only from a distance not exceeding 2 inches from the shell. It is, therefore, necessary to know the rate of water exchange within the narrow layer adjacent to the oyster bottom. The situation may be different in the case of vertical mixing of water due to turbulent flow.

The amount of water available to an oyster population can be calculated if the number of individuals on the bottom and the rate of water movements are known. In the case of a turbulent flow, vertical mixing of water depends on the degree of turbulency. If the mixing extends to the height of 1 foot above the mud line, the total volume of water in which the oyster population lives in our example is 1 acre-foot or 325,851 gallons (1.25 million l.). It follows that the current velocity must be strong enough to renew the volume of the layer above three times (3.75÷1.25) every hour or 72 times in 24 hours. In cases of greater population density the current requirements are proportionally higher. Due allowance should be made, of course, for the presence of other water-filtering animals which compete with the oyster for food. It is clear, therefore, that great concentrations of water-filtering organisms are possible only where there is sufficient renewal of water. The oyster reef in the Altamaha Sound, Ga., (fig. 367) is a good example of this condition. Such concentrations of live oysters crowded over a limited space cannot exist in sluggish water and are found only in rapid tidal streams.

The water movement factor can be evaluated by determining whether the rate of renewal of water over the bottom is sufficient for the needs of the population and whether the pattern of circulation is such that a certain percentage of

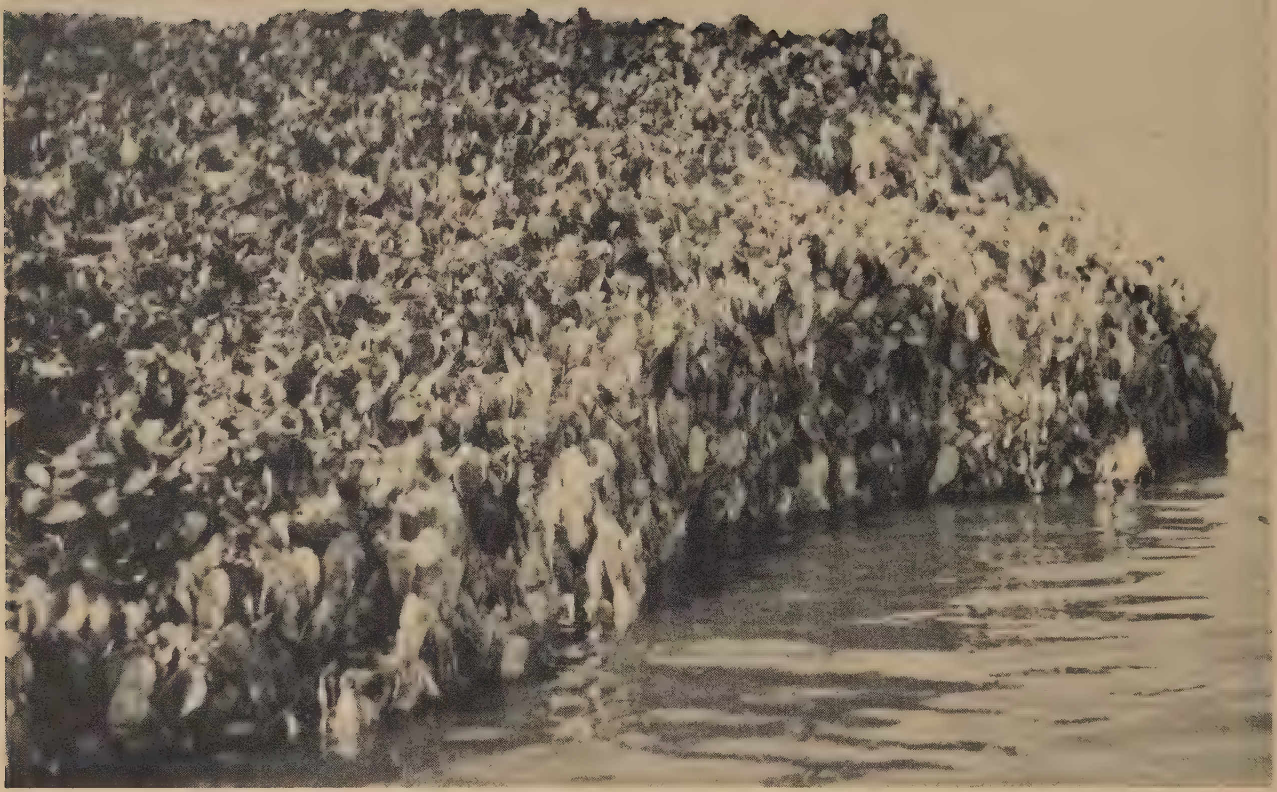

Figure 367.—Oyster reef in Altamaha Sound, Ga., at low tide, March 1925. The highest point was about 6 feet above the bottom; water at the foot of the reef was 8 inches deep. The reef consists of live oysters growing on the side and upper surfaces and attached to empty shells.

oyster larvae will be retained in the estuary by the end of the larval period. It is obvious that the requirements of water movements for the growing of oysters are different from those necessary for the settlement of larvae.

SALINITY

The general rule that the composition of sea water is constant and varies only in the degree of dilution by fresh water is applicable to the estuaries and other basins which have direct communication with the sea. Only in exceptional cases is the circulation in an estuary so impeded that stagnation and oxygen deficiency develop and render the area unsuitable for oyster growth and reproduction.

Oysters like many other euryhaline organisms are able to live in sea water of very wide range of salinity. According to the so-called Venice system of classification of saline waters adopted at the symposium organized by the International Association of Limnology and the International Union of Biological Sciences at Venice, Italy, in April 1958, the range of salinity favorable for *C. virginica* falls within two zones, the polyhaline, from 30‰ to 18‰, and the mesohaline, from 18‰ to 5‰ (Symposium for the Classification of Brackish Waters, 1958). Populations of oysters found beyond the upper or lower limits of the range exist under marginal conditions. Their growth and gonad formation are inhibited, and they are often decimated either by floods, in the lower zone of the range, or by predators which usually remain in more saline waters. The noncommercial oyster of the Atlantic and Gulf coasts, *O. equestris*, prefers more saline waters and has been found on buoys as far as 20 miles offshore where surface salinities ranged from 33‰ to 36‰ (Galtsoff and Merrill, 1962). In the subtidal regions of the coastal waters this species is found in salinities of 20‰ to 25‰.

An unstable salinity regime is an important ecological factor in the tidal rivers and streams inhabited by *C. virginica;* diurnal, seasonal, and annual fluctuations are the normal features of such an environment. Their effect on *C. virginica* depends on the range of fluctuations and the suddenness of the changes. For instance, oysters that were replanted in September from a low salinity area of the upper Chesapeake Bay (10°/₀₀ to 12°/₀₀) to the high salinity water of Sinepuxent Bay (32°/₀₀ to 33°/₀₀) all perished within 3 to 4 weeks after planting. Examination of the new grounds disclosed that the sudden change in salinity during hot weather was the primary cause of mortality. On the other hand, a similar transfer made by the same grower succeeded in October and November when the air and water temperatures were much lower.

The mean values of diurnal, seasonal, and annual salinities are of little significance for evaluating their effect on an oyster population. The oyster can isolate itself from the outside environment by closing its valves tightly and survive adverse conditions, provided they do not last indefinitely (see: ch. VIII). Since changes in salinity are commonly associated with temperature changes, an attempt was made by Hedgpeth (1953) to combine the two factors to express what he calls "hydrographic climate." He plotted ranges of means and extremes or monthly means of salinity against temperature and obtained polygons which provide a graphic representation of the conditions existing in a given area for the indicated period. The method appears to be useful and may be profitably applied to oyster research.

Oysters inhabiting the parts of estuaries in which salinity is below 10 °/₀₀ are seriously affected by fresh water and could be destroyed by floods lasting for several weeks. Mobile Bay, Ala., investigated in 1929, may be cited as an example of this condition. Oysters in Mobile Bay grew on reefs which extended from the upper to the lower parts of the bay. The river discharge into the bay normally resulted in a salinity gradient from 5°/₀₀ to 30°/₀₀. However, in the 36 years from 1893 to 1929 the two tributaries, the Tombigbee and Selma Rivers, rose 27 times to flood stage with the flood conditions lasting from 4 to 31 days. The height of the rivers at flood stage in February and April 1929 was 65.4 and 56.2 feet, respectively, and lasted for 32 days (Galtsoff, 1930) with the result that fresh water prevailed over almost the entire bay and the mortality at different parts of the bay varied from 100 percent in the upper parts to 85 and 54 percent at the passes to Mississippi Sound.

Oysters in Mississippi Sound often suffer from long-continued low salinities. Mortality of oysters in the Sound occurs when the local precipitation in the Sound area, in the Pearl River basin, or at some more distant point in the Mississippi River basin occurs more or less simultaneously and lowers the salinity to a harmful level (Butler, 1949b, 1952).

Freshets sometimes kill oysters in the James River, Va. During a 6-week period from May 1 to June 15, 1958, many of the native oysters died, and as many as 90 percent perished on some grounds where salinity did not become suitable until July 1 (Andrews, Haven, and Quayle, 1959). In a test made at the Virginia Fisheries Laboratory at Gloucester Point, oysters held in trays in low salinity areas were "conditioned" to a low physiological state (absence of heart beat and ciliary motion and loss of mantle sensitivity). The investigation lead to the conclusion that oysters conditioned slowly at low temperatures and low salinities can endure a prolonged situation of unsuitable environment. Andrews, Haven, and Quayle infer that "the mechanism of conditioning appears to be a type of narcotization," an interesting idea which, however, needs verification.

The first symptoms displayed by an oyster affected by water of lowered salinity are partial or complete contraction of the adductor muscle and slowing or cessation of water current through the gills. With the drop counting technique described in chapter IX it can be shown that the ciliary activity of the gill epithelium immediately decreases when it comes in contact with water of lowered salinity. The effect may be brief or prolonged, depending on the degree of change from the salinity level to which the oyster had been adapted. When the salinity change is about 10°/₀₀ and continues for several hours, both the rate of water transport and the time the oyster remains open are decreased, and under extreme conditions the feeding and respiration cease. Experimental studies on the adaptation of oysters to salinity changes were first made by Hopkins (1936) on *C. gigas* of the Pacific coast. He recorded the changes in the opening and closing of the valves, and registered the deflection of a small plate placed in front of the cloacal current. As indicated in chapter IX this method is not reliable for a quantitative determination of the volume of water transported through the gills but is adequate for determining the relative strength of the cloacal current. The results show that the adaptation to new conditions depends upon the degree of change. Recovery was more rapid when the salinity was increased than when the same degree of change was made in the opposite direction. At a salinity of about 13°/₀₀ very little water was transported even after several days were allowed for adaptation, but recovery to normal activity followed rapidly after the return of the oyster to a normal environment in water of 26°/₀₀ to 29°/₀₀. Increased salinity, from 25°/₀₀ to 39°/₀₀, produced no significant changes in the water transport by *C. gigas*. An unfavorable effect was recorded at 56°/₀₀, which is considerably above the normal range of the oyster's habitat.

In experiments at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn. (Loosanoff, 1952), *C. virginica* from Long Island Sound accustomed to water of a stable salinity of about 27 °/₀₀ were placed directly in water of 20, 15, 10, and 5 °/₀₀ made by the addition of a corresponding amount of fresh water. The loss of food caused by the addition of plankton-free water was compensated by providing measured amounts of phytoplankton culture. The decrease in the rate of water transport was proportionate to the degree of change and varied from 24 to 99.6 percent of the normal rate. Six hours of exposure to the lowest salinities tested resulted in no permanent injuries, and within a few hours after transfer to the salinity of their natural habitat the oysters fed, reformed the crystalline styles, and discharged true feces and pseudo feces. Other experiments at Milford at the same time demonstrated that oysters conditioned to live in low salinities can tolerate lower concentrations of salts than oysters living in more saline waters. Although the oysters were observed to feed in water of 5 °/₀₀ salinity their shell movement and water transport were abnormal and growth was inhibited.

The reproductive capability of oysters is inhibited by low salinity. Butler (1949a) showed that this is due primarily to the failure of gonad development in oysters of the marginal area of upper Chesapeake Bay; his findings were confirmed by experiments with Long Island Sound oysters (Loosanoff, 1952). These experiments have not demonstrated whether the failure of gonad development is the direct result of lowered salinity or is due to inadequate feeding.

Long-continued exposure to salinities above the 32°/₀₀ level also has an unfavorable effect on oyster populations. This can be seen from the conditions of Texas oyster beds. During the 6-year drought from 1948 to 1953, the salinity in the bays of the central Texas coast generally rose well over 36°/₀₀ and at times reached the 40°/₀₀ level without an appreciable decrease in the winter (Parker, 1955). Previous records, from 1922 until 1948, show that during most of the year salinity in this area ranged from 5°/₀₀ to 25°/₀₀ with somewhat higher salinities in the summer. With the increase in salinity there was a gradual replacement of *C. virginica* populations by *O. equestris*. In 1952 over half of the young oysters (spat) were *O. equestris*, whereas in years of low salinity the reefs were comprised primarily of *C. virginica*. It is not known whether the observed change was due to the inhibition of gonad formation or to the failure of oyster larvae to reach setting stage. From an ecological point of view it is, however, significant that the replacement of one species by another took place at the time of the increase in salinity of water. The surviving *C. virginica* were observed to develop different shell characteristics: the valves became crenulated, thin, sharp, and highly pigmented.

Under certain circumstances the influx of fresh water into estuaries may be beneficial. Some of the carnivorous gastropods, flatworms, and starfishes, which are highly destructive to oysters, are killed by brackish water that constitutes a barrier through which they cannot penetrate. Decrease in the salinity of water protects the populations of oysters at the heads of the bays. Periodical flushing wipes out the predators and restores the productivity of beds. The population of oysters in areas highly infested by their enemies, as in the Apalachicola Bay, the upper half of the Delaware Bay, and many others, cannot exist if the access of fresh water to oyster bottoms is diminished and the salinity increases above the 15°/₀₀ level.

The evaluation of the salinity factor can be estimated by determining the total percentage of time the brackish water of less than 10°/₀₀ or water of salinity exceeding 34°/₀₀ remains on an oyster bottom. The zero value is assigned to conditions unsuitable for the oyster's existence; marginal conditions are indicated by 1, and optimum conditions by 10. The values between are based on the percentage of time the oyster population is affected by unfavorable salinities.

TEMPERATURE

A great difference in climatic conditions exists within the range of distribution of *C. virginica.* The water temperature under which the species lives varies from a minimum of about 1° C. during the winter in northern states to a maximum of about 36° C., which occasionally has been observed in Texas, Florida, and Louisiana. The temperature of oysters exposed to the sun at low tide on the flat registers 46° to 49° C. measured by inserting a small thermometer between the slightly opened valves. Normally oysters of the tidal zone remain exposed for 2 to 3 hours at the maximum. Occasionally strong offshore winds drive the waters away and oysters beds in shallow places remain out of water for several days. Such instances occur along the coast of Texas where as a rule the low stage of water is caused by strong northern winds. The exposed population may perish either from excessive warming or from freezing temperatures brought from the north by cold fronts.

The temperature regime affects the life of the oyster by controlling the rate of water transport, feeding, respiration, gonad formation, and spawning. *C. virginica* ceases feeding at a temperature of 6° to 7° C. The maximum rate of ciliary activity responsible for the transport of water is at about 25° to 26° C.; above 32° C. ciliary movement rapidly declines. Nearly all functions of the body cease or are reduced to a minimum at about 42° C. Using the seasonal fluctuations of temperature, it is easy to determine the percentage of time during which oysters in any given locality continue to feed and reproduce. Similar observations may be made on the growth of shells and calcification. Two curves in figure 368 show the seasonal changes in mean monthly temperatures in two localities separated by about 11.5 degrees of latitude. The northern location of oyster grounds of Long Island Sound is at about lat. 41° 30′ N.; the southern location is that of Apalachicola Bay, Fla., at about lat. 30° N. The two curves, upper for Apalachicola and lower for Long Island Sound, parallel each other but are at two distinct levels. The difference is greatest during the winter and early spring and is smallest during the fall. The two temperature levels indicated by broken lines mark the periods of successful mass spawning and setting of oysters at temperatures of 20° C. and above and the inhibition of feeding and growth of the oysters below 8° C., often called hibernation. Apalachicola oysters continue to feed and grow throughout the year, and their reproductive season may last for 7 months or 58 percent of the year, whereas the period of feeding and growth of the northern oysters is limited to about 6½ months, or 56 percent of the time and the reproductive season is restricted to 2 summer months, or about 16 percent of the time. The use of monthly means based on several years of observations gives a picture of a general condition not unduly influenced by short-term fluctuations which may differ from year to year.

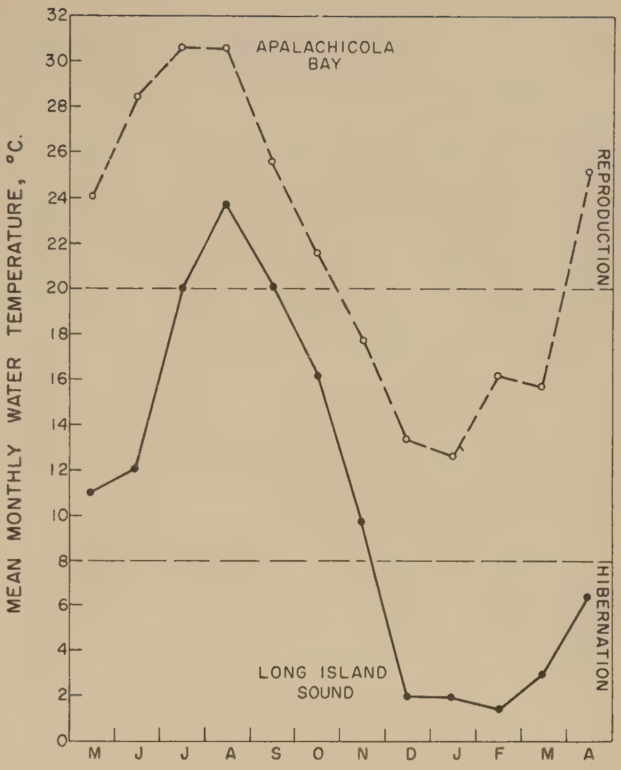

FIGURE 368.—Mean monthly temperature in °C. of water in Apalachicola Bay (upper curve) and Long Island Sound (lower curve).

Little is known about the prolonged effect of temperatures above 32° to 34° C. on oyster populations. From a few physiological observations it may be inferred that long continued exposure to high temperature is unfavorable and impedes the normal rate of water transport by the gills.

The percentage of time available to an oyster population for growth and "fattening", or for reproduction, can be used in evaluating the effect of the temperature factor on the productivity of an oyster bottom. A distinction should be made between the reproductive capability of the population and its growth and "fattening". In the practice of oyster culture the areas of bottom most suitable for setting are not considered desirable for the rapid growth and conditioning of oysters for market and vice versa.

FOOD

The quantities of food available to water-filtering animals may be determined by taking plankton and nannoplankton samples and by noting the food requirements of a given species. It has been shown by Jørgensen (1952) and Jørgensen and Goldberg (1953) that the oyster (*C. virginica*) and the ascidians (*Ciona intestinalis* and *Molgula manhattensis*) filter about 10 to 20 l. of water for each ml. of oxygen consumed, and that about two-thirds of the energy absorbed by them can be used for growth. The actual food requirements of the animals studied by Jørgensen probably do not exceed 0.15 mg. of utilizable organic matter per liter of water used. Determinations of phytoplankton in American coastal waters made by Riley (1941), Riley, Stommel, and Bumpus (1949), and Riley and Gorgy (1948), show that the organic matter of the phytoplankton in their samples ranged from 0.17 to 2.8 mg. per liter. These waters contain enough material to supply the energy requirements of *C. virginica* which, according to my determinations, differ from those made by Jørgensen (see p. 210 in ch. IX); under normal conditions at 24° to 25° C. *C. virginica* uses from 3 to 4 mg. of oxygen per hour.

Quantitative samples of plankton and microplankton taken throughout the year from the water over a thriving oyster population can be compared with samples collected in the plankton-poor waters of the tropics. The water should be pumped from the bottom zone, with care being taken not to stir the sediment. Vertical hauls are useless since the water a few inches above the oysters does not come in contact with them except in the case of strong vertical mixing.

Seasonal changes in the volume of plankton and microplankton of water over a commercially productive oyster bed in Long Island Sound are shown in figure 369. Both types of samples were collected at the same time. For the plankton study 50 l. of water were filtered through No. 20 bolting silk. The collected material was preserved in 2 percent formalin and transferred into tall glass cylinders, and its volume read 24 hours later after the material had settled at the bottom; the results are expressed in cm.^{3}. For microplankton determination a 1 liter sample was taken from the bottom and the water passed through a high-speed Foerst-Juday type centrifuge rotating at 20,000 r.p.m. The centrifugate was transferred to a 15 mm. diameter tube and centrifuged for 5 minutes in a clinical centrifuge at 14,000 r.p.m. and its volume measured. Since the waters of Long Island Sound are relatively free of silt, the amounts of detritus

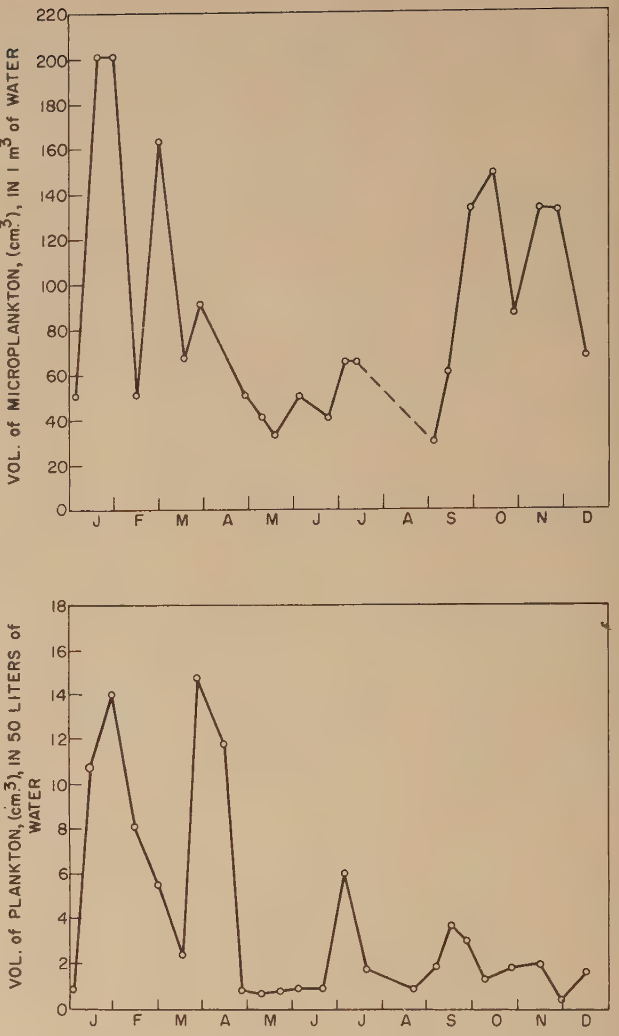

FIGURE 369.—Seasonal changes in the volume of plankton (upper curve) in cm.^{3} per 50 l. and microplankton (nannoplankton) in cu. cm. per 1 m.^{3} (lower curve) in Long Island Sound in 1932 and 1933.

in the samples were insignificant. Seasonal fluctuations of plankton and nannoplankton follow a typical pattern of maximum development from about the middle of January to the end of April, followed by a decrease during May to September and a second maximum, smaller than the first, in September to November. The latter period is of greater significance for the northern oysters because they do not feed during the winter months of January and February.

Another method of determining plankton productivity of water over an oyster bottom was used in a study of the York River, Va. (Galtsoff, Chipman, Engle, and Calderwood, 1947). In this case the water sample was obtained by means of a Birge-Juday plankton trap. Two liters of the sample were passed through the Foerst-Juday centrifuge, and the centrifugate extracted for 15 minutes in 10 ml. of 80 percent acetone. A comparison of the color was then made with standards of nickel sulfate and potassium dichromate (Harvey, 1934), and the results expressed in pigment units per liter of original sample. Each unit was found to be equivalent to approximately 10,000 diatoms and dinoflagellates present in York River water. So far as seasonal changes in plankton are concerned, the results were similar to those observed in Long Island Sound. Although the total energy requirements of certain filter-feeders are known, no information is available about the specific food that is needed for their growth and reproduction. Certain types of phytoplankton, such as *Chlorella*, have antibiotic properties and are harmful to some bivalves. Too little is known about the specific food requirements of various organisms. The total bulk of phytoplankton may be made of the materials of low nutritive value or may consist of such organisms as *Rhizosolenia*, *Chaetoceras*, and others which cannot be ingested by the oyster because of their size and shape.

In evaluating the food factor the abundance of plankton should be compared in different areas during the period when the oyster is actively feeding and accumulating glycogen. This period usually occurs shortly after spawning (ch. XVII). The amount of phytoplankton per unit of volume of water on oyster bottoms of highest commercial yield and consisting primarily of algae that can be utilized by the oyster represents the optimum value of food factor (score 10); while the phytoplankton content of water from the marginal areas is assigned the score 1.

Another and more accurate method may be used. It is based on the determination of metabolic rate of oysters during the period of feeding. The nutritive value (food energy) of the sample of phytoplankton can be determined by using a bomb calorimeter and measuring the heat of combustion in calories. Knowing the rate of water transport by the oyster at a given temperature and salinity, it is easy to calculate whether the food supply on oyster grounds is adequate. Unfortunately, the method widely used in nutritional studies has not yet been applied in oyster research.

The high concentrations of phytoplankton which occur during blooms are not desirable features and can be harmful. Experimental work has clearly shown that at a certain high concentration of several forms (*Nitzschia closterium*, *Prorocentrum triangulatum*, *Euglena viridis*, and *Chlorella* sp.) the rate of water transport of oysters is reduced and feeding ceases (Loosanoff and Engle, 1947). The deleterious effect is caused by the cells themselves and by their metabolites. These laboratory findings are in accord with field observations in Great South Bay, N.Y., where a mass development of a *Chlorella* like organism adversely affected valuable oyster beds. Another example of a danger of excessive development of a single microorganism is the so-called red tide (Galtsoff, 1948, 1949) along the western coast of Florida. Sudden development of the dinoflagellate, *Gymnodinium breve*, causes extensive mortality of fishes and kills many oysters growing along the shores of the affected area.

Conditions are ideal for the feeding of oysters when water free of pollution and containing a low concentration of small diatoms and dinoflagellates runs over a bottom in a nonturbulent flow.

NEGATIVE FACTORS OF ENVIRONMENT

The environment itself may interfere with the welfare of oyster populations. Negative factors decrease or inhibit reproductive capabilities; destroy the population by causing extreme adverse conditions; increase the incidence of disease; inhibit the fattening and the growth of oyster body, thus decreasing the productiveness of an oyster bed; and interfere with the formation of shell and so deprive the oysters of their principal means of protection against adverse situations and attacks of enemies. All negative factors are evaluated by determining the degree of their harmfulness and assigning them scores from 1, for 10 percent effectiveness, to 9, for 90 percent. The score 10, indicating 100 percent destructiveness, is omitted because no oyster population can exist under such a condition. Zero score means complete absence of a negative factor.

SEDIMENTATION

Rapid settling of suspended material may be highly destructive to an oyster community. All coastal waters contain a certain amount of solids in suspension of either organic or inorganic origin. The particles settle on the bottom, depending on their weight and shape, chemical composition, temperature, viscosity of water, and character of water movements. The velocity of the fall of a particle through a liquid is a function of the radius of the particle governed by Stoke's law:

$$v=\frac{2(P_0-P)gr^2}{gn}$$

where v is the velocity of sinking, P_o and P are the densities of the particle and of the liquid respectively, r the radius of the particle, n the absolute viscosity of the liquid, and g the acceleration of gravity. It is assumed in the equation that the particles are spherical, that they are small enough so that the viscosity of the water is the only resistance to their fall, and that their sinking is not impeded by adjacent particles. The equation is applicable to spheres varying in size from 0.2 to 200μ and suspended in quiet water. Obviously such conditions represent the "ideal" situation which cannot be found in an estuarine environment. Here the water is in almost constant motion with rapid changes in the direction and velocity, and carries sediments consisting of particles of differing sizes, shapes, and densities. The discussion of the physical aspects of sedimentation problems is beyond the scope of this book. The reader interested in these problems is referred to comprehensive textbooks on the subject (Twenhofel, 1961; Linsley, Kohler, and Paulhus, 1949). For an understanding of the ecological effects of sedimentation it is enough to describe in general terms the conditions under which silt particles are transported and deposited on estuarine bottoms.

Observations made on waterflow in a tube show that at low velocities the particles of a liquid move in parallel lines and the resistance to their motion is due to viscosity. This condition is called streamline or laminar flow. High velocity of the water and roughness in the walls of the tube make the stream break into a turbulent flow characterized by irregular, eddying, and rolling movements. The formation of the eddies counteracts the gravitational settling of particles, and more material is moved upward than sinks toward the bottom. When the amount of sediment picked up by a turbulent flow exceeds the amount deposited, the bottom is eroded. If the gravitational force predominates, more material is being deposited than carried away and the bottom is rapidly covered by the sediment. In an estuary both processes alternate following the rhythmic changes in the direction and velocity of tidal currents. In many instances an equilibrium is established and continues for a long period of time unless the balance between the two forces is upset by violent water movements from severe storms or floods. Depending on the configuration of the bottom, certain areas of an estuary are scoured, while vast quantities of sediments are deposited on others. This is typical on oyster grounds in many tidal streams.

The material suspended in coastal water is a very complex mixture of particles of differing size, shape, specific gravity, and mineralogical composition. The particles are sorted out as they are moved by the water. According to Trask (1950, p. 10), slowly moving water seems to make for poorer sorting than a fast moving current. To a sedimentation geologist the resulting pattern of

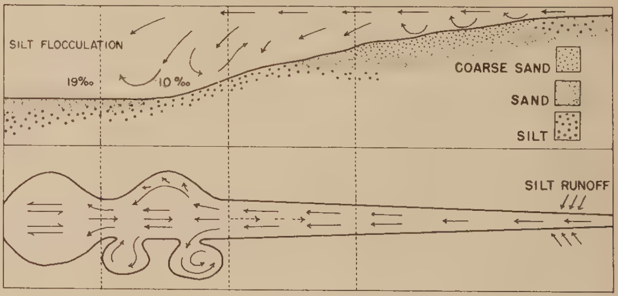

Figure 370.—Diagrammatic representation of the sorting out and deposition of coarse sand, fine sand, and silt in an estuary. The lower part of the diagram indicates the complex system of current and counter current. From Rochford, 1951.

the size distribution of sediment may serve as an indication of the mode of its deposition. A schematic representation of sorting of the material suspended in water and its deposition in the four zones of an estuarine system according to the distance from the mouth of the river is shown in figure 370, redrawn from Rochford (1951). At the head of an estuary silt is transported by tidal streams further than sand and because of flocculation, caused by the influx of saltier water, smothers the beds near the mouth (middle and left parts of fig. 370). The complexity of the horizontal pattern of currents is shown on the lower part of the diagram.

Particles immediately above the bottom move by rolling, sliding, and jumping. Outside of this narrow "bed layer" (Einstein, 1950) the particles in water constitute the suspended load; their weight is continuously supported by the fluid until they reach the lower part of the estuary where they are deposited over the bottom and on tidal flats.

The problem of transport and settling of sediments in the sea is not well understood (Trask, 1950, p. 9) and is being intensively studied by many oceanographic institutions of the world. In tidal waters where oyster beds are located the problem becomes more complex because the rate of transport and settling is greatly influenced by periodic changes in current velocities, turbulence, salinity, temperature, density and viscosity of water, and size, shape, roughness, and specific gravity of transportable particles. In the tidal regime where salty oceanic water mixes with fresh water another important factor called flocculation enters into this already complex picture. Small dispersed particles of clay and of organic detritus have a tendency to aggregate in small lumps. In the purification of drinking water flocculation is produced artificially by adding a coagulant such as aluminum sulphate $(Al_2(SO_4)_3 \cdot 18\ H_2O)$. In estuaries where sea and fresh water mix the flocculation is caused by the change in the electric charges of the particles which occur with the change in the hydrogen ion concentrations.

Laboratory experiments with kaolin suspension show that flocculation of particles about 4μ in diameter may occur at constant pH (8.5) by increased concentrations of Na^+ at constant SO_{4--} concentrations (Whitehouse, 951, 11952).[9] The results indicate the tendency of positive ions to cause the flocculations of the negatively charged particles.

Flocculation in coastal waters may be observed from the deck of a ship at the time of freshets along the coastline of the Carolinas and Georgia. Large patches of aggregated silt particles show clearly in the salty offshore water while the brownish color of less saline water at the mouth of a river remains uniform.

Rochford (1951) states that flocculation of silt takes place in the salinity zone of less than 19‰ and higher than 10‰ (fig. 370). Since flocculation depends on several factors including temperature, pH, and the type of sediments in suspension, one may expect a great variation in the rates of settling in the various estuaries of the Atlantic and Gulf coasts.

The gross effect of sedimentation may be measured by determining the depth of deposition of silt over an oyster bottom per unit of time and area. Less noticeable, but highly significant, is the deposition of a thin layer of sediment over the hard surfaces to which many organisms, including oyster larvae, attach. A deposit of loose sediment only 1 or 2 mm. thick is enough to make the surface of shells and rocks unsuitable for the attachment of larvae and to cause failure of setting. I have observed such conditions many times in certain sections of Oyster River, Chatham, Mass.; in the Wiweantic River of the Cape Cod area; in the small rivers and creeks emptying into Delaware Bay; and in the Rappahannock and York rivers, Va. Undoubtedly similar conditions may be found in many other places where silt is transported by estuarine currents. Light sedimentation is not harmful to populations of adult oysters, but may be heavy enough to interfere with their reproduction.

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[9] WHITEHOUSE, U. GRANT.

1951. A study of chemical sedimentation and of physical oceanography sponsored at Texas A. and M. College by the American Petroleum Institute through the Scripps Institution of Oceanography of the University of California (A.P.I. Project 51). Progress report for the quarter ending December 31, 1951. The Texas A. and M. Research Foundation, Project 34, The Agricultural and Mechanical College of Texas, Department of Oceanography, College Station, Texas, 9 pp. (Preliminary report).

1952. A study of the chemistry of oceanic sedimentation sponsored at Texas A. and M. College through the Scripps Institution of Oceanography of the University of California (A.P.I. Project 51). Progress report for quarter ending December 31, 1952. The Texas A. and M. Research Foundation, Project 34. The Agricultural and Mechanical College of Texas, Department of Oceanography, College Station, Texas, 11 pp. (Preliminary report).

Many formerly productive oyster bottoms along the Atlantic Coast of the United States have been destroyed by a high rate of sedimentation. Dead oyster reefs buried below a surface of mud in the waters of Louisiana and Texas are good examples of this process. The silting of estuaries may be studied by the simple method of comparing the depth of water shown in navigation charts of 25 or 50 years ago with present soundings. From these differences the total amount of deposit accumulated over the given period of time can be computed. The reduction of volume of water in a basin due to sedimentation can be determined from these data and from the computations of the capacities of reservoirs (Dobson, 1936). Brown, Seavy, and Rittenhouse (1939) successfully used this method in determining the rate of silting over a distance of 19.4 miles of the York River, Va. In 1857 the water volume of this sector of the river, estimated at mean low tide, was 227,780 acre-feet. By 1911 it was reduced to 222,189 acre-feet, and in 1938 was only 206,896 acre-feet. The cumulative volume of sediment deposited during the period 1857–1911 was 5,591 acre-feet, and from 1911 to 1938 reached 20,884 acre-feet. The annual deposition for the first period of 55 years was 104 acre-feet, which represented 0.05 percent loss of water volume; during the second period of 27 years (1911 to 1938) the annual deposition increased to 566 acre-feet, which corresponded to an annual 0.25 percent loss of water. The increased rate of silting during the later period was explained by an increased erosion of soil over the watershed resulting from faulty agricultural practice, deforestation, and an increase in population.

The filling of bays and estuaries with sediments is a general phenomenon along the Texas coast, and is particularly pronounced in Laguna Madre and near the mouth of the Colorado River in Matagorda Bay where some of the buried oyster reefs are found under 14 feet of mud (Norris, 1953). During the last 36 years silting has destroyed 6,000 to 7,000 acres of productive oyster reefs near Matagorda. These beds were described in detail by Moore (1907) and resurveyed in 1926 by Galtsoff (1931a). In 1926 the principal reefs opposite the mouth of the Colorado River were surrounded by very soft mud but were still productive. Now the mud of the Colorado River has completely buried these reefs and pushed the head of fresh water seaward. For the Neuces River, a small stream emptying into Corpus Christi Bay, the accumulation of silt recorded by comparing the depths given on 1880 charts with those issued in 1937 varied from 4 feet near the ship channel to less than 1 foot at the south shore (Price and Gunter, 1943). The average annual accumulation of sediment near the ship channel was about 0.8 inches, only slightly less than the annual increase of oyster shell in height. Under such conditions an individual oyster, even if it grew in the vertical position that oysters usually assume in soft mud, in 6 to 7 years would have sunk in mud for about three-quarters of its height and perished.

The soft, muddy tidal flats typical for the inshore waters of the South Carolina and Georgia coast are usually devoid of oysters. U.S. Bureau of Fisheries experiments on oyster farming in 1939 and 1940 in the vicinity of Beaufort, S.C., demonstrated the complete unsuitability of these areas for oyster culture. Reinforcement of these flats by shells planted in a layer about 1-foot thick and strong enough to support a man's weight was a complete failure. The shells acted as baffles, with the result that in a short time mud filled all the crevices between them and in about 6 weeks completely covered everything with a smooth layer of silt (Smith, 1949). Similar results were obtained with brush and other materials placed on the surface of the mud; in a few months not a trace of them could be seen on the surface. On the other hand, oysters grew well along the opposite side of the river where a swift current kept the bottom scoured.

Accumulation of silt over an oyster bottom is sometimes caused by the activity of various mud-gathering and mud-feeding invertebrates. Chief among them are several species of the mud worm, *Polydora*. The two long antennae of these worms protrude from the tube in which the animal lives and sweep the surrounding water. Mud particles suspended in the water are caught by the epithelium of the antennae and by ciliary motion are transported toward the head to accumulate around the worm's body and thus making the tube. Some of the mud is ingested and passes through the intestime. *P. websteri* invades the shell cavity of the oyster, settles on the inner surface at a right angle to the edge, and builds a U-shaped mud tube with both orifices external. The structure is soon covered by a layer of conchiolin deposited by oyster and becomes a semitransparent blister.

As the worm grows the cavity it occupies is enlarged by boring to provide for its increased size. Several shell layers are deposited by the oyster over the blister. The mechanism of boring is not well understood; probably erosion of the shell is accomplished by a combined chemical and mechanical action. *P. ligni* is found living in mud tubes on tidal flats or attached to shells and rocks. On several occasions the reproduction of *P. ligni* on the oyster bottoms of Delaware Bay was so rapid that nearly every live oyster of the affected area was killed by a deposit of mud several inches thick consisting of numberless live worms and their tubes. The process of gathering mud by *Polydora* is shown in a photograph (fig. 371) taken of a live worm, which was placed in a glass tubing for observation in the laboratory.

Oysters themselves accumulate large quantities of organic sediment, which is discarded with the feces. During feeding the oyster discharges fecal ribbons at the rate of several centimeters per hour. In a sluggish current large quantities of fecal masses settle in the crevices between the oysters and contaminate the bottom. The situation may become serious enough to cause a decline in the productivity of oyster beds, as has been demonstrated by Japanese biologists (Ito and Imai, 1955).

Organic material constitutes a major portion of marine muds. The physical properties of a sediment may be of lesser importance to oyster ecology than the complex biochemical changes associated with the bacterial decomposition of its organic components that result in the formation of carbon dioxide, ammonia, phosphates, sulfates, and various organic acids. In the case of anaerobic oxidation, methane and hydrogen sulfide are formed (Waksman, 1942; Waksman and Hotchkiss, 1937). The effect of these products of decomposition on bottom populations probably is the main reason for the slower rate of growth for oysters on the bottom than for those which are kept above the bottom on trays or are suspended from rafts and floats (Shaw,

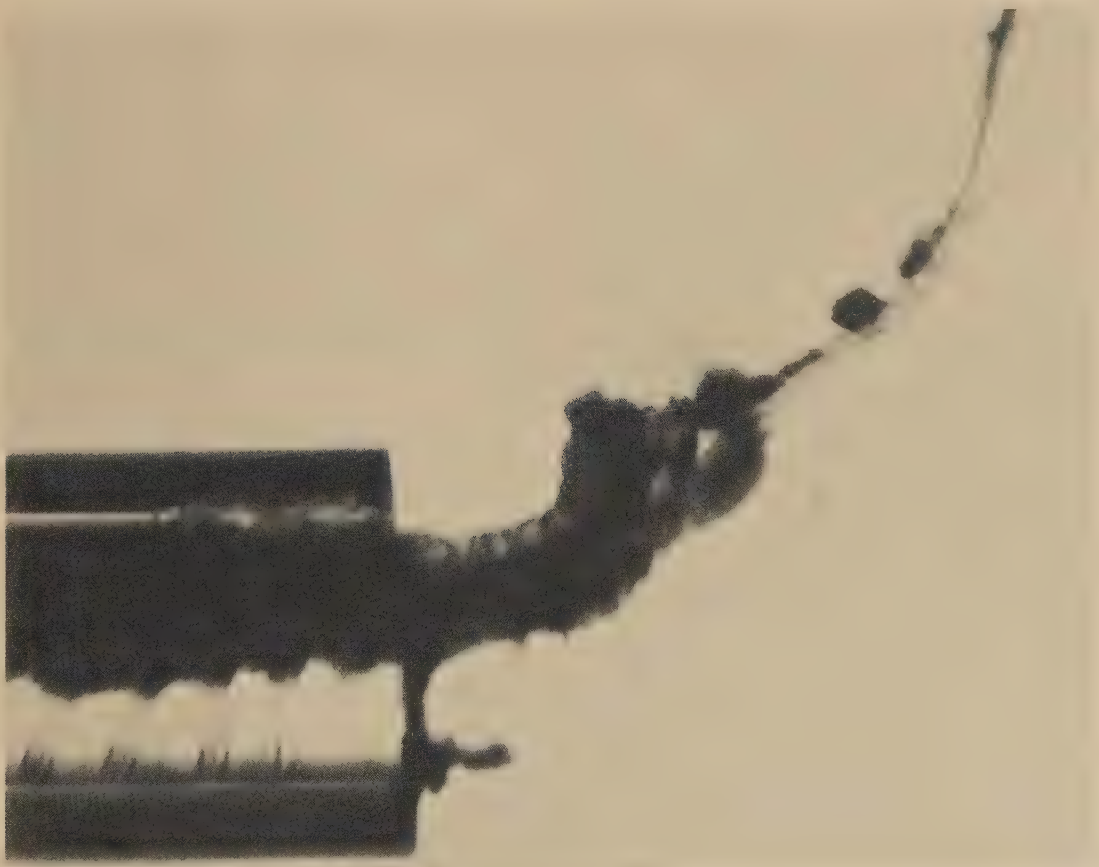

FIGURE 371.—Photograph of live *P. ligni* collecting mud. The worm is contained in a glass tube placed in sea water containing silt in suspension. Mud particles along the grooved antennae are transported toward the mouth and have begun to accumulate at the edge of the glass tubing.

1962). It appears paradoxical but true that the conditions of a natural environment do not add up to the ideal situation for the life of a bottom inhabiting mollusk such as the oyster. Location on rocks and underwater structures above the mud line appears to offer a more favorable environment.

Determination of the amount of silt settling on the bottom can be made with a mud trap of the type shown in fig. 372. The trap consists of a metal funnel riveted to a metal frame and a quart size container (fruit jar) screwed to the funnel. The trap is set on the bottom for the desired length of time, then carefully lifted, and the amount of sediment settled over the area of the funnel and inside the jar is measured.

Data obtained with a mud trap of the type shown above measure the rate of settling of sediments on the bottom. One should bear in mind, however, that a certain portion of the sediment deposited during slack tide may be washed away as the current velocity increases with tidal changes. Furthermore, mud already settled on the bottom may be stirred by wave action and resettle on an adjacent area. The actual accumulation of sediment can be measured with a mud board of the type shown in fig. 373. A wooden stake 36 inches high supports a flat board 18 inches long and 6 inches wide, mounted horizontally, and the stake is forced into mud so that the board is level with the bottom. This type of trap can be used conveniently on tidal flats.

A trap to be used below low water can be made of a flat board ½ square yard in area. To prevent the loss of sediment the board has slanted borders about 4 inches wide, projected above its upper surface. The trap is set on four short legs; its surface is ruled in squares to facilitate the measurements of the area covered with sediment. The thickness of the sediment is measured with a ruler. Since the board cannot be lifted without disturbing and losing the accumulated sediment, observations must be made by a diver.

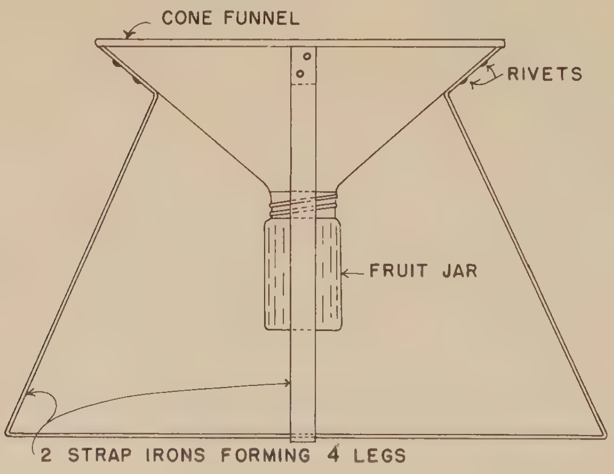

FIGURE 372.—Mud trap for collecting sediments settling from water.

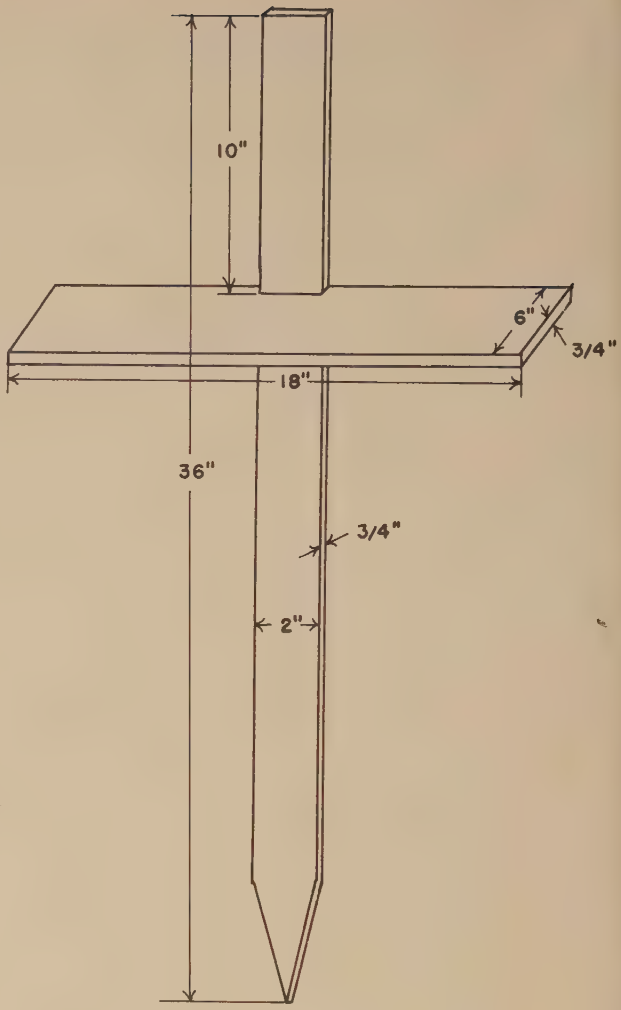

FIGURE 373.—Mud board for measuring the accumulation of sediment on tidal flats.

An indirect estimate of the amount of material in suspension can be made by using a Secchi disc and recording the depth of extinction of white color. The results are affected by the visual acuity of the observer, illumination, and the condition of the sea surface.

The amount of suspended material in a sample of water can be determined with a simple turbidimeter of the type used primarily in fresh-water studies; more accurate results are obtained with an electrophotometer and spectrophotometer. The turbidity of the collected samples is compared with standards made of known dilutions of a suspension of 1 g. of kaolin (or silica clay) in 1 l. of water.

Evaluation of the effect of sedimentation on oyster bottoms can be made by considering the location of oyster bottom in the estuary; the amount and character of sediment in suspension; the type of estuarine circulation; and the rate of accumulation of sediment on oysters. A score of zero is assigned to the ideal conditions under which no deposits settle on live oysters. The opposite extreme, valued at 10, is found in the areas of heavy sedimentation, not suitable to oysters. All intermediate conditions scored from 1 to 9 can be evaluated on the basis of field observations.

DISEASE

Oysters suffer from both noncontagious and infectious diseases. The first category is associated with the malfunction of physiological systems of organs and deficiencies in the environment, such as lack of food, unsuitable salinity and water temperature, and pollution by domestic sewage and various trade wastes. The second category, infectious diseases, is caused by pathogens and parasites. Clear distinction between the two types of pathological conditions is not always possible because resistance to infection is lowered by an unfavorable environment, and an oyster weakened by adverse conditions more easily succumbs to infection.

With few exceptions the outward symptoms of a disease are nonspecific. The more common symptoms are slow growth, failure to fatten and develop gonads, recession of the mantle, and valves that remain slightly agape. There is often a corresponding abnormal deposition of shell material that in a chronic condition causes the formation of short and thick shells ("huître boudeuse" of French biologists). The valves do not close tightly because the adductor muscle is weakened. The body of a sick oyster is watery, often discolored (dirty green and brown), and bloody with blood cells accumulating on the mantle and on the surface of the gills.

The etiology of oyster diseases is not well known. A few microorganisms infecting the oyster have been definitely identified as pathogens; the taxonomic position of others is not known, and some are called by code numbers. Oyster populations throughout the world suffer from periodic widespread mortalities which may be associated with infections, but since the life cycles of some of the pathogens have not been described, the evidence remains circumstantial.

The widespread mortality of oysters rarely can be attributed to a single factor of the environment; in most cases it occurs as a result of the combination of several adverse conditions including infection.

Malpeque Bay disease

One of the most persistent and mysterious ailments of oysters is the Malpeque Bay disease, which in 1915 and 1916 struck the populations of *C. virginica* in the bays of Prince Edward Island, Canada, causing the death of 90 percent of the oysters, and in later years appeared along the Canadian mainland. The most distinctive symptom associated with the disease was the occurrence of yellow-green pustules, up to 0.5 cm. in diameter, on the surface of the visceral mass, along the edges of the mantle, and on the adductor muscle and the heart. Despite lengthy field and experimental studies conducted from the epidemic year to the present, the causative agent has not been found, although there is no doubt that the mortality of Malpeque Bay oysters was due to an infection (Needler and Logie, 1947). With the expectation that the survived oysters were of disease resistant stock, the Department of Fisheries of Canada and the Fisheries Research Board organized in 1957 a rehabilitation project and transferred oysters from Prince Edward Island to devastated mainland areas. Unfortunately the hopes did not materialize fully since a high proportion of the spat that settled on the rehabilitated area did not show the expected level of resistance. It is hoped, however, that a resistant stock will develop from a small number of survivors over a period of several years (Drinnan and Medcof, 1961).

Dermocystidium marinum

Dermocystidium marinum Mackin, Owen, and Collier, a fungus of uncertain taxonomic position infecting *C. virginica*, is probably the most dangerous pathogen associated with periodic mortalities of oysters in the waters of southern States. The microorganism infects oyster tissues producing single, spherical, vacuolate cells which reproduce by endogenous free cell formation and subsequently liberate uninucleate sporelike bodies. The following detailed description of the species is reproduced verbatim from Johnson and Sparrow (1961, p. 539):

> Mature thallus a hyaline, spherical, spore-like body, 2–30μ, averaging about 10μ in diameter; each cell containing a large, slightly eccentric vacuole in which a polymorphic, refractive vacuoplast usually occurs; nucleus oval, eccentric; cleaving internally to form a short hypha terminated by an apical, conidium-like swelling.

Dermocystidium can be identified on cross-sections of an oyster stained with hematoxylin, Giemsa or other histological stains, or in teased preparations stained with Lugol solution (fig. 374).

Identification of *Dermocystidium* by microscopic examination of tissues is time consuming and difficult. The diagnostic technique developed by Ray, Mackin, and Boswell (1953) facilitates the examination of large numbers of samples. Small pieces of tissues are removed from the gaping oysters and placed in Carrel tissue culture flasks containing a small amount of sterile water to which streptomycin and penicillin have been added to prevent bacterial growth. Prior to excision the tissues are washed in sterile sea water, then placed for 10 minutes in a 10 percent solution of sodium merthiolate (1:10,000), washed again in sea water, and allowed to remain for several hours in sterile sea water fortified with 1,000 units each of streptomycin and penicillin. Tissues parasitized with *Dermocystidium* disintegrate completely in about a week, while in the controls they remain intact. The debris of disintegrated tissues consists mainly of minute spheres of *Dermocystidium* cells. Unfortunately the contamination of samples with molds, yeast, and ciliate protozoans could not be entirely prevented and failures were "much more frequent than were successes, and most of the experiments were discarded." The data presented by Ray, Mackin, and Boswell show that the major effect of *Dermocystidium* infection is marked loss of weight, averaging 33 percent.

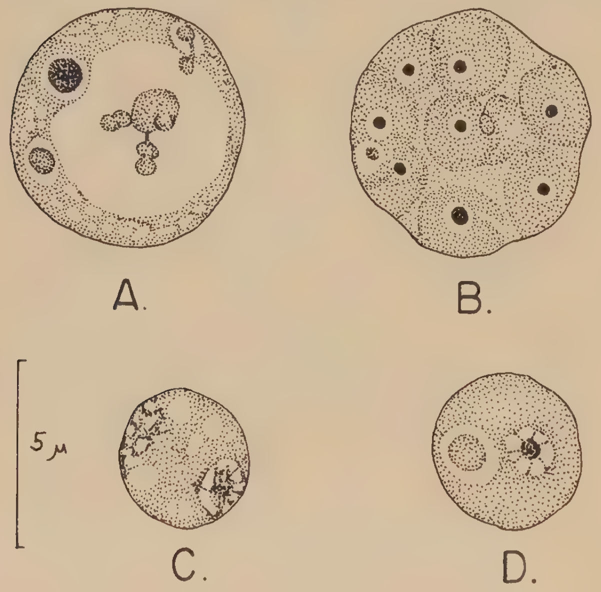

FIGURE 374.—Drawings of *Dermocystidium marinum* stained with Heidenhain's iron hematoxylin and eosin. A—A mature spore with markedly irregular vacuoplast, cytoplasmic inclusions, and very large vacuole. B—Multiple fission resulting in several daughter cells. C—A binucleate stage with chromatin in diffuse condition, and showing beginning vacuolation of the cytoplasm. D—An immature spore with small vacuole and vesicular nucleus. Figure 1 from Mackin, Owen, and Collier, Science, vol. 111, No. 2883, 1950, p. 329.

The infection may combine with other factors to produce a mortality of oysters which, according to Mackin (1961a), can virtually destroy seed oysters planted in Louisiana in a single summer. *Dermocystidium* studies in southern waters established the significant fact that the effect of the parasite "is not only a matter of disease but of season, summer losses accruing from disease being significantly greater than those of early spring months" (Ray, Mackin, and Boswell, 1953). The importance of environmental factors (temperature and salinity) is clearly demonstrated by these findings.

According to Mackin (1961b), the disease caused by *Dermocystidium* affects the oysters from Delaware Bay to Mexico but in the more northerly part of the range is not apparent in winter. It is not clear, however, if *Dermocystidium* remains in a dormant stage or if it disappears from oysters. In the Gulf States winter temperatures are probably not low enough to eliminate the parasite, and consequently considerable mortality may occur in mild winters.

Dermocystidium marinum and possibly other species of the genus have been reported from *O. frons*, *O. equestris*, *O. lurida*, *Mya arenaria*, *Mulina lateralis*, *Macoma baltica*, *Mercenaria* (*Venus*) *mercenaria*, *Anadare transversa*, *Anomia simplex*, *Ensis minor*, *Laevicardium mortoni*, and *Lyonsia hyalina* (Johnson and Sparrow, 1961, p. 540).

Many phases of the life history and biology of *Dermocystidium* require elucidation, particularly the transport of spores by water and their penetration into the tissues, the details of reproductive cycles, the relationship between environmental conditions and degree of infection.

Disease associated with *Haplosporidium*

Excessive mortality of oysters in Delaware Bay in a 6-week period of April and May 1957 wiped out from 35 to 85 percent of planted oysters and almost completely ruined the oyster industry of the State. A microorganism consistently found in tissues of infected oysters was designated by the code name MSX and later on was tentatively identified by Mackin as one of the Haplosporidia. The organism invades the connective tissue surrounding the intestine and digestive diverticula. Early plasmodial stages and ensuing stages of development are shown in two illustrations (figs. 375 and 376) made in the laboratory from a preparation kindly supplied by Haskin.

Mortality of oysters on the eastern shore of Virginia near Seaside was investigated from 1959 to 1961 by the Virginia Institute of Marine Science. The microorganism causing the disease and first designated as SSO was described by Wood and Andrews (1962) as a sporozoan, *Haplosporidium costale*, n. sp., infecting connective tissues of oysters and producing a truncate spore encased in an operculum with a lid. An early plasmodium with 6 to 12 nuclei is from 6 to 8μ in size (fig. 377). *Haplosporidium* has been found in live oysters as early as February, and in mid-May to June the infection may cause high mortality. How the parasite infects the oysters is not known, and its life history is not fully understood (Andrews, Wood, and Hoese, 1962).

Shell disease

This disease, which is probably associated with an unidentified fungal infection of oyster shell, is not particularly serious in *C. virginica*, but has been reported to cause catastrophic mortalities in the population of *O. edulis* in Oosterschelde, Holland. The disease can be recognized by bottle-green or orange-brown rubberlike warts and spots

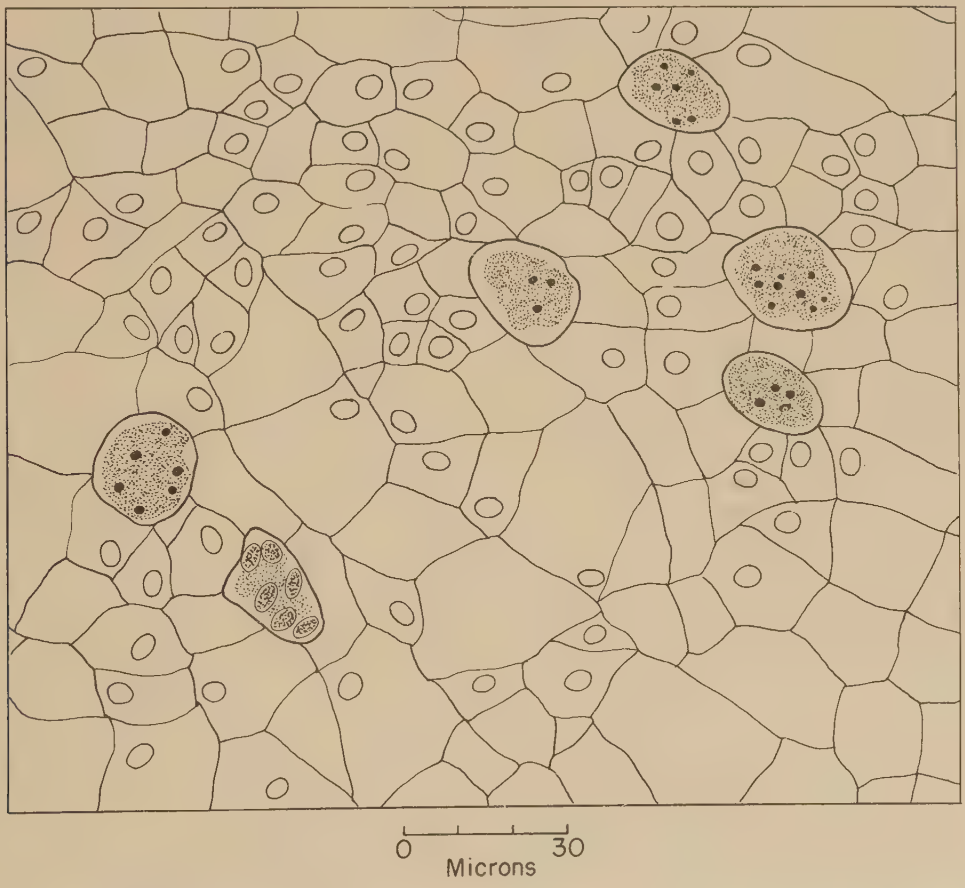

FIGURE 375.—Plasmodial stage of MSX in the connective tissue of heavily infected *C. virginica* from Delaware Bay. Bouin, hematoxylin-eosin.

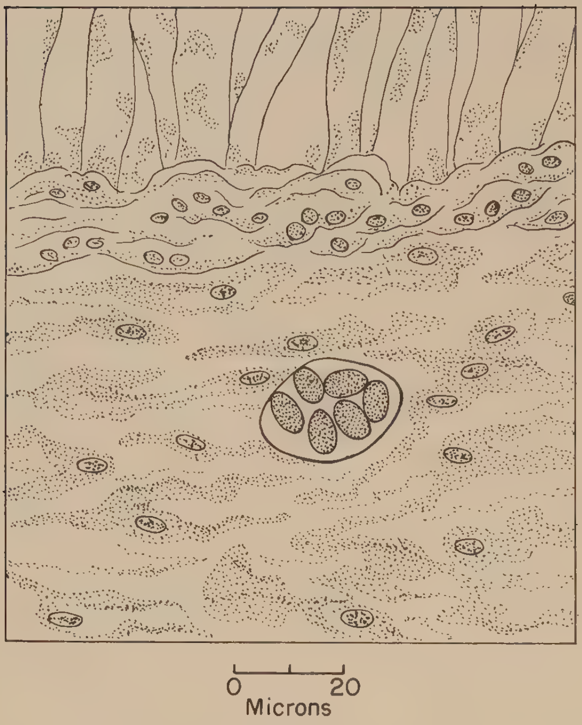

FIGURE 376.—Later stage of development of MSX in the connective tissue of heavily infected *C. virginica* from Delaware Bay. Formalin 10 percent, iron hematoxylin.

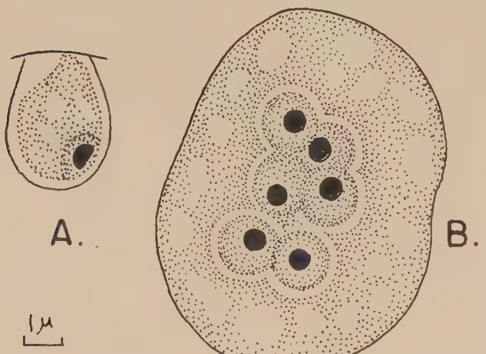

FIGURE 377.—*Haplosporidium costale.* A—mature spore; B—early plasmodium. From Wood and Andrews, fig. 1, Science, vol. 136, 1962, p. 711.

on the inner surfaces of the shell and, in more advanced cases, by deformation of the shell edges and hinge. Examination of thin slides of shell show abundantly branching fungus. The shell disease in Oosterschelde was studied by Korringa (1951a), who discovered that it spreads at water temperatures above 19° C. and that the higher the temperature the more vigorous the attack. The fungus was not isolated from Dutch oysters and remains unidentified. Korringa believes that it survives in the old green cockle shells scattered as cultch over the bottom and that its spores are probably carried by the water currents. Wholesale cleaning, removal of old shells, and disinfecting of young infected oysters with a solution of "an organic salt of mercury" (not fully specified by Korringa) are recommended as control measures.

Shell disease in Dutch oysters has been known since 1902, but at that time occurred only in a limited percentage of oysters. Its rapid spread in the years following 1930 was probably due to the enormous quantities of old cockle shells, about 40,000 to 50,000 m.^{3}, scattered annually as spat collectors. This gave the fungus a chance to proliferate more rapidly and infect the oysters. Voisin (1931) describes the disease in oysters imported from Zeeland, Holland, for planting in the Marennes area on the west coast of France. He states that more than 40 percent of these oysters had shells infected by a fungus, probably belonging to the genus *Monilia.* The identification is merely a guess and cannot be verified.

Foot disease

Foot disease or "maladie du pied" of French oyster growers occurs in *O. edulis* and *C. angulata* in the waters of the western and southern coasts of Europe. Korringa suggests that it is probably identical with the shell disease. The name is an obvious misnomer because the foot is lacking in all adult oysters.

"Foot disease" has existed in the Arcachon region since 1877. Giard (1894) described its parasitic nature and attributed it to a schizomycete fungus *Myotomus ostrearum* Giard, a genus not listed in Johnson and Sparrow's treatise on fungi (1961).

The disease affects the area of the attachment of the adductor muscle, primarily on the lower, concave (left) valve, and in certain cases the upper, flat valve. The surface of the shell under the muscle is covered with small, rough dark green spots. In advanced cases the muscle becomes detached from the valve and forms irregular cysts of horny and slightly elastic material. Later on when the cyst extends beyond the area of the muscle attachment, the cyst walls become covered with calcareous shell deposit. According to Giard (1894) and Dollfus (1922), the parasitic fungus grows by utilizing the conchiolin of the shell and stimulates its secretion by the mantle.

The progress of the disease is slow. During the advanced stage shell movements are affected and the oyster has difficulty in closing its valves, thus becoming an easy prey for its enemies.

Foot disease is found in *C. virginica*, particularly in oysters inhabiting muddy waters of the southern States, but in my experience it never reaches epizootic proportions. The cysts of an affected oyster (fig. 378) contain a suspension of blood cells, debris, and numerous bacteria which probably represent secondary infection. The disease does not present a serious menace to the oyster fishery of the coastal states.

Hexamita

The flagellate *Hexamita inflata* was first found in the intestinal tract of *O. edulis* (Certes, 1882). It is present in *C. virginica* of Prince Edward Island, Canada, and southern Louisiana, and in *O. edulis* in Dutch waters (Mackin, Korringa, and Hopkins, 1952). Heavy infection with *Hexamita* causes breakdown of connective tissue cells, general inflammation, the appearance of many trophozoites in blood vessels, and necrosis of adjacent tissues. The early stages of the disease appear to be intracellular, and are usually found in the leucocytes of the blood vessels. The trophozoite is oblong and narrow at the anterior, with six anterior and two posterior flagella. Cysts found in advanced stages of the disease are small, about 5 μ in diameter; they contain two or four small nuclei and have no flagellar structure. The complete life cycle of the parasite has not been described. The method of infection appears to be by cysts liberated after the disintegration of an infected oyster body. Experimental studies by Stein, Denison, and Mackin (1961), who used diseased *O. lurida*, give no evidence that *Hexamita* is a highly pathogenic parasite because there was no significant difference in the mortality between the experimental and control specimens.

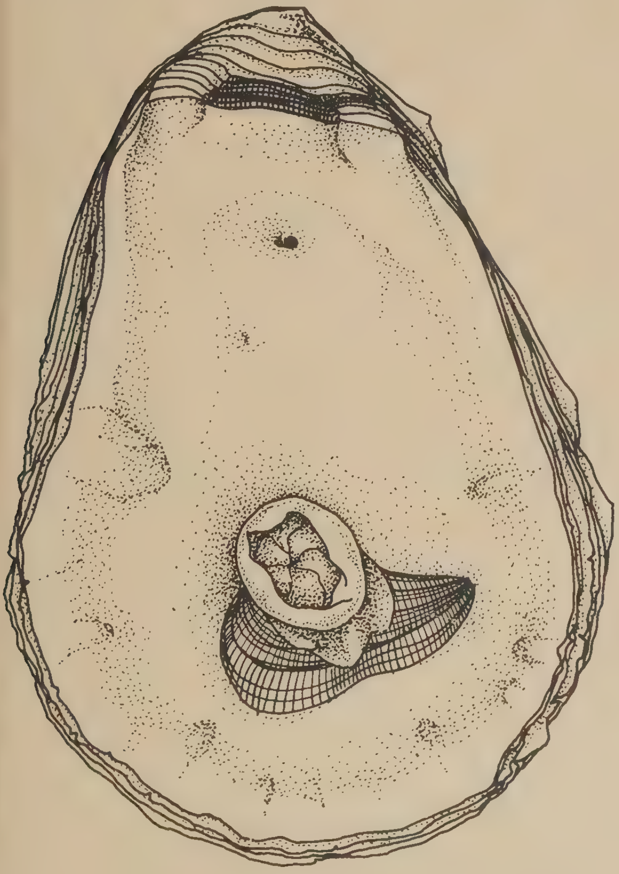

Figure 378.—Large cyst filled with blood cells, bacteria, and debris of muscle tissue in *C. virginica*.

Nematopsis

Cysts of the gregarine *Nematopsis* are frequently found in the tissues of several European bivalves including *O. edulis*, *Mytilus*, *Cardium*, *Donax*, *Tellina*, *Mactra*, *Solen*, and others (Dollfus, 1922). Observations by Louis Léger (quoted from Dollfus) showed that vegetative stages of the gregarine are often found in the kidneys and that the spores with sporozoites are usually located in the gills. Léger also showed that the intermediary hosts are the crabs *Carcinus moneas* and *Portunus depurator*. *Nematopsis* develops in the intestinal canal of the crab and forms cysts which are rejected into water and are transmitted with water currents. There was no evidence that *Nematopsis* is pathogenic.

The species *N. ostrearum* from *C. virginica* has been described by Prytherch (1940), who found the parasite in the oysters of Virginia, North Carolina, and Louisiana. He expressed the belief that mortality of oysters in Virginia and Louisiana was directly caused by this gregarine. *Nematopsis* is widely distributed throughout the waters from the Chesapeake Bay states to Louisiana. Its distribution indicates no correlation with oyster mortalities in that area (Landau and Galtsoff, 1951).

Trematodes and parasitic copepods

The trematode, *Bucephalus haimeanus* Lac. Duth., is occasionally found in *O. edulis* and *C. virginica*. According to Tennent (1906), who studied its life history, the worm thrives in oysters of brackish water and is inhibited by an increased salinity. In cases of heavy infestation, the gonads and digestive diverticula are almost completely replaced by cercariae and by the long germ tubes of the sporocysts, which after their liberation infest *Menidia*, other small fishes, and *Tylosurus marinus*. Destruction of the gonad is the most obvious pathological effect caused by *Bucephalus*. So far this trematode has not been suspected of causing mortalities in oyster populations.

The parasitic copepod *Mytilicola intestinalis* is common among mussels of the Mediterranean. Another species, *M. orientalis*, infests *C. gigas* and *Mytilus crassitesta* of the Inland Sea of Japan. The parasitic copepod is found in the intestinal tract of bivalves and is easily recognized by its red color and relatively large size which makes it visible to the naked eye. In the United States *Mytilicola orientalis* is widespread in lower Puget Sound, occurring in *O. lurida* and *C. gigas*, *Mytilus edulis*, *Paphia staminea*, and *Crepidula fornicata*. Infection is heaviest in the common mussels, often reaching 100 percent in some areas (Odlaug, 1946). A single specimen of *Mytilicola intestinalis* was found by Pearse and Wharton (1938) in *C. virginica* on the Gulf coast of Florida. The presence of *M. orientalis* in *O. lurida* in the lower Puget Sound area interferes with their fatness, but apparently inflicts no serious injuries to oyster stocks. In *C. gigas* the copepod produces metaplastic changes in the gut, completely destroys the ciliated epithelium, and penetrates the underlying connective tissue (Sparks, 1962).

The presence of parasites in adult oysters makes them unmarketable for esthetic reasons and, therefore, detracts from the commercial productivity of oyster bottoms.

Any disease factor, regardless of the identity of the pathogen, can be evaluated by determining the percentage of the infected oysters, the intensity of infection, the loss caused by the mortalities, and the decrease in yield of marketable oysters.

COMMENSALS AND COMPETITORS

The shell and body of the oyster are the natural abodes for many plants and sedentary animals which attach themselves to the shell surface or bore through it to make for themselves a well-protected residence; some settle on the soft body without penetrating its tissues while others invade the inner organs. The difference between the commensals, i.e., organisms which share the food gathered by the host, and the parasites, which live at the expense of their hosts and sometimes inflict serious injuries, is not very sharp. Some commensals may cause injury to the host and become parasites.

Competitors are those organisms which live in close proximity to each other and struggle for the space and food available in the habitat. Some appear to be innocuous while others by virtue of their habits and high reproductive capabilities are harmful.

Boring Sponges

Small round holes on the surface of mollusk shells indicate the presence of the most common animal associated with the oyster, the boring sponge. There are seven species of the genus *Cliona* along the Atlantic Coast of the United States. In a case of heavy infestation the shell becomes brittle, breaks under slight pressure, and reveals conspicuous tunnels and cavities filled with yellow sponge tissue. Microscopic examination shows a typical sponge structure with numerous siliceous spicules from 150 to 250 μ long, of the type called tylostyles, and small skeletal elements of different shapes and sizes known as microscleres. Species identification is based on the type of cavities or galleries made by the sponge and the shape and sizes of the spicules (Old, 1941). Small fragments of shell material at the holes by *Cliona* may suggest mechanical action of the sponge. Warburton (1958) found experimentally that sponge cells in contact with a surface of calcite form a reticulum of fine pseudopodia and filaments. A corresponding pattern of lines is etched into the mineral, and the marked areas are of the same size and shape as the fragments discharged by the sponge. Apparently the cytoplasmic filaments penetrate the calcite by secretion of minute amounts of acid and undercut fragments which are carried out by excurrent canals of the sponge.

It is not known whether boring sponges use the organic component (conchiolin) of the shell, but it is obvious that they do not draw their nutrients from the body of the oyster. The sponge touches the surface of the body only in cases of old, heavy infestation. In such instances the holes made by the sponge are rapidly covered by a deposition of conchiolin. The holes made by the sponge are clearly visible on the inner surface of the valve under a newly deposited layer of conchiolin (fig. 379). The race between the sponge and the oyster

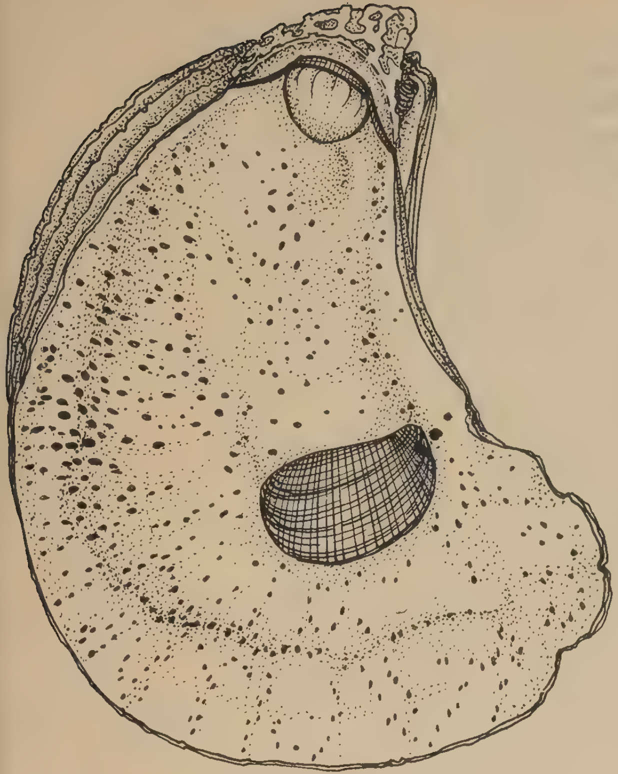

FIGURE 379.—Left valve of adult *Crassostrea virginica* heavily infested by boring sponge, *Cliona celata*. Woods Hole.

continues, and in most cases the oyster's protective measures prevent direct contact between the sponge and the mantle. However, should the deposition of shell material be delayed by adverse conditions, the sponge makes direct contact with the mantle and produces lysis of the epithelium and underlying connective tissue. Dark pigmented pustules form exactly opposite the holes in the shell. This extreme case observed in oysters kept for several months in the laboratory is shown in fig. 380. The tissue of these oysters is flabby, and the mantle is easily detached from the shell surface.

All oyster bottoms are, to a certain degree, infested by boring sponges which are found in both live oysters and empty shells. There are certain areas, however, where the infestation is particularly heavy and the growth of the sponge is very rapid. After the death of an oyster the sponge continues to grow on the shell, forming large, irregular masses 2 or more feet wide and several inches thick. About 30 years ago such large specimens were common in the bays and harbors of southern Cape Cod, but now they are found only in deep offshore waters. The effect of the boring sponge can be estimated by determining the percentage of oysters with heavily infested and brittle shells and by comparing their solid and glycogen contents with those of uninfested oysters.

Boring clam

Oyster shells in the south Atlantic are often infested with a boring clam, *Diplothyra smithii* Tryon of the family Pholadidae. Many papers on oyster biology refer to this clam as *Martesia* sp., but the taxonomy of the family revised by Turner (1955) corrects the nomenclature and restricts the name *Martesia* to wood-boring clams.

The boring clam *D. smithii* is about one-half inch long. It is usually found inside the shell material in a cavity which increases in size with the growth of the clam. The range of distribution extends from northern Cape Cod (Provincetown, Mass.) south to the east and west coasts of Florida, Louisiana, and Texas. I have found no live clams in oyster shells during my long-continued studies in New England waters, and only a few live specimens have been recovered from dead oyster shells around Tangier Sound in the Chesapeake Bay. In southern waters the boring clam is very common, particularly on some reefs on the Texas coast. In 1926 oysters from Matagorda Bay, Tex., were found to be so heavily infested by *Diplothyra* that over 200 clams of various sizes were found in a single adult (fig. 381). In order to make this count the shell was dissolved in hydrochloric acid and the bodies of the clams were collected.

As the cavity bored by the clam increases and approaches the inner shell surface, the oyster protects itself by depositing layers of conchiolin over the nearly perforated areas. Very rarely does one find an oyster in which there is a direct contact between the clam and oyster mantle. On the outer surface of the shell the presence of clams is indicated by small holes. The weakening of the shell structure is the main effect of the boring clam on the oyster.

Mud worms

Of the several species of Polydora found in the intertidal zone of the Atlantic and Pacific coasts of the United States, only two, *P. websteri* Hartman and *P. ligni* Webster, are important to oyster ecology. *P. websteri* is found in oyster shells and on the inner surfaces near the valve

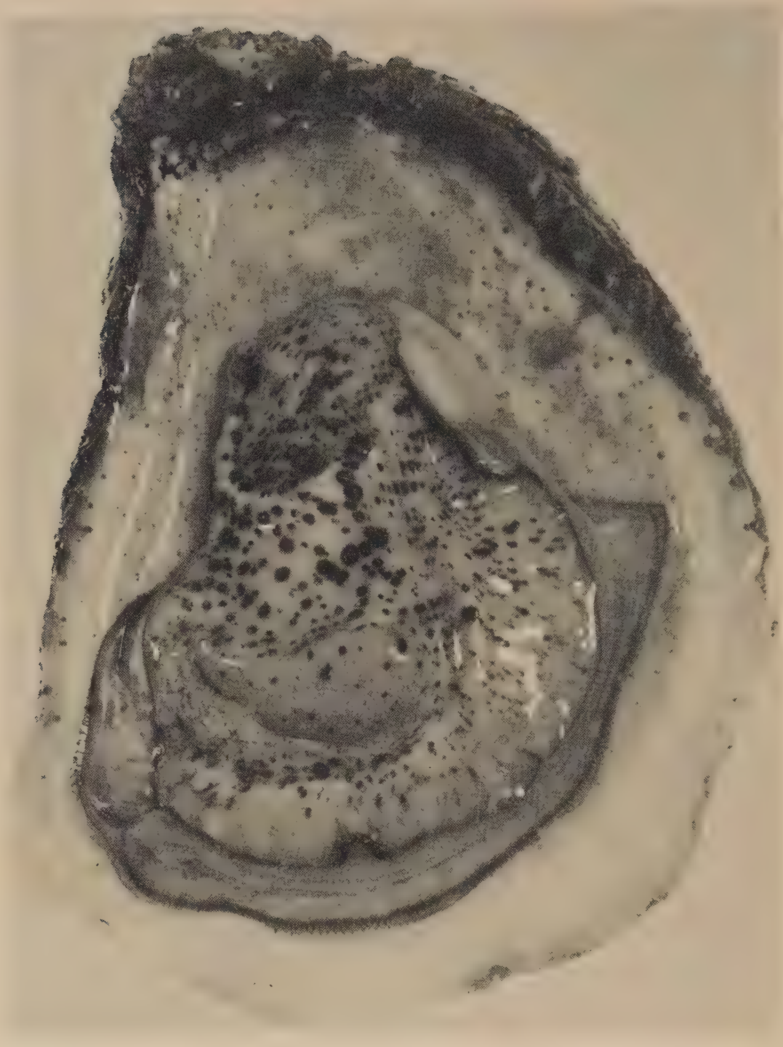

FIGURE 380.—Black pustules on the surface of the visceral mass and mantle of *C. virginica* caused by contact with boring sponge, *Cliona celata.* Photograph of an oyster kept in the laboratory tanks at Woods Hole.

edges. The worm accumulates mud and builds a U-shaped tube which is covered by semitransparent shell material secreted by the oyster. The formation is usually called a blister. *P. ligni* is abundant on tidal flats where it can be found living in small mud tubes or in crevices of waterlogged wood structures and other submerged objects. The mud worm may be indirectly destructive to oysters, for when many worms settle on shells they can smother an entire oyster population with their tubes. *P. ciliata* (Johnston) has been accused of extensive mortalities of oysters in New South Wales, Australia (Roughly, 1925). Frequent reports of finding this species on the coast of eastern America are based on erroneous identifications and probably should be referred to as *P. websteri* (Hartman, 1945). Korringa (1951b) finds no serious injuries by *P. ciliata* to oysters (*O. edulis*) in Dutch waters and thinks that in many areas the damages were caused by *P. websteri* and *P. hoplura.*

Knowledge of the life histories of *P. websteri* and *P. ligni* is incomplete. Both species lay eggs in capsules attached to the inner walls of the tube in which the animal lives. The egg-laying was noticed in the Woods Hole laboratory when *P. ligni* were placed in small glass tubing of appropriate length and diameter (fig. 382). The process of egg laying has never been observed in spite of frequent examination of several tubes during both day and night (Mortensen and Galtsoff, 1944). However, egg capsules were found attached to the walls of the tubes shortly after *Polydora* were left undisturbed in darkness.

The eggs develop within the capsule until the larvae have acquired three pairs of setiferous segments; then they leave the tube. At a temperature of 21° to 23° C. the development of *P. ligni* under laboratory conditions varied from 4 to 8 days. Larvae of *P. websteri* (fig. 383) also have three setiferous segments. According to Hopkins (1958), planktonic larvae, presumably *P. websteri*, occur in Louisiana waters throughout the year; eggs were found in the tubes when water temperature ranged between 12° and 18° C.

The duration of the pelagic life of either species of *Polydora* is not known. The planktonic larvae grow and develop additional segments before they settle on the substratum. Since the largest *P. websteri* worm found in plankton had 17 segments and the smallest found on oysters also had 17 segments, it is probable that this species settles at that age. The appearance of young *P. ligni* at an early bottom stage is shown in fig. 384.

The larvae of *P. websteri* settle on the rough exterior surface of young oysters and make shoe-shaped burrows near the extreme edge of the valves. As the worm grows it enlarges its burrow. The process of excavation is probably chemical, apparently similar to that described by Wilson (1928) for *P. hoplura* and by Hannerz (1956) for *P. ciliata*.

The tubes of *P. ligni* are made of mud particles held together by mucus secreted by the antennae and the body surface. Ciliary motion along the tentacle grooves serves as an efficient mud-gathering device. Experimental evidence shows that if the lumps of mud are too large or if particles consist of the finest sand or foreign materials such as corn starch or powdered glass, the ciliary motion is reversed and the material is rejected. These laboratory observations prove that the worm is capable of selecting the substances needed for the building of a soft tube.

The tube inhabited by the worm, whether U-shaped or straight, is lengthened by the worm at both ends. To accomplish this *P. ligni* reverses its position in the tube by folding itself halfway and sliding over its own ventral side. The process, frequently observed in the Woods Hole laboratory, is accomplished with great speed and remarkable ease.

The amount of mud which *P. ligni* can accumulate in the formation of their tubes is astonishing. A sample collected on June 8, 1944 from the tidal flats of Delaware Bay contained about 430 closely packed worm tubes per square inch of mud area. They all lay nearly perpendicular to the surface. A cubic inch of the washed and dried sample weighed 20 g., of which 12.8 g. consisted of mud with the balance made up of sand, empty shells, and organic matter. On this basis it is estimated that the worms gathered 4.9 pounds of dry mud

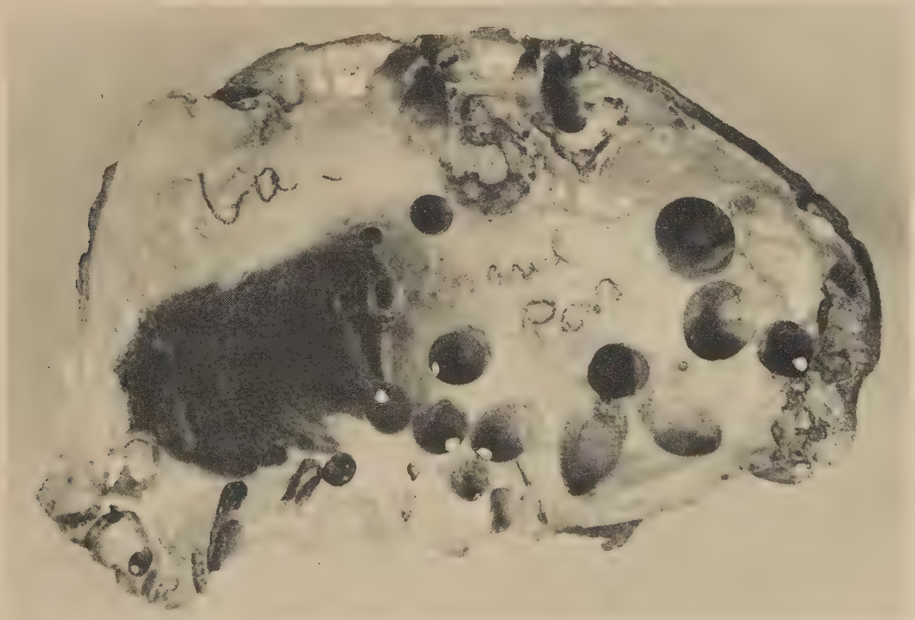

FIGURE 381.—Photograph of an adult *C. virginica* from Matagorda Bay, Tex., heavily infested with *D. smithii*. The outer layer of the shell was chiseled off to expose the cavities.

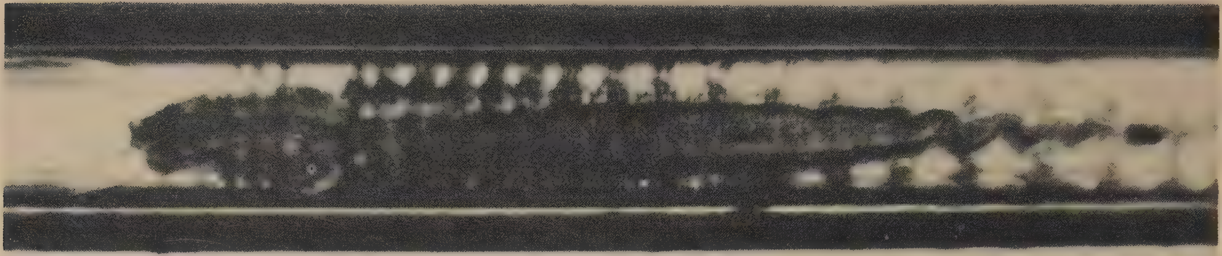

FIGURE 382.—Photograph of live *P. ligni* lying quiescent inside a glass tube. Dorsal view.

per layer of surface 1 square foot in area and 1 inch deep.

Since *P. websteri* is confined in oysters to mud blisters and does not come in direct contact with oyster tissues, it causes no visible injuries. This view is corroborated by the observations of Loosanoff and Engle (1943), who found that oysters heavily infested with *P. websteri* and grown in trays above the bottom were in excellent condition.

However, personal observations made in Seaside, Va. and in Texas bays convinced me that oysters heavily infested by mud worms (fig. 385) are usually in poor condition. This opinion is shared by Lunz (1940, 1941), who calls the mud worm a pest in South Carolina oysters. According to his observations, 20.9 percent of the oysters growing on the hard surface of tidal flats are infected, and the percentage increases to 51.9 on soft, muddy bottoms above low-water mark. There is no

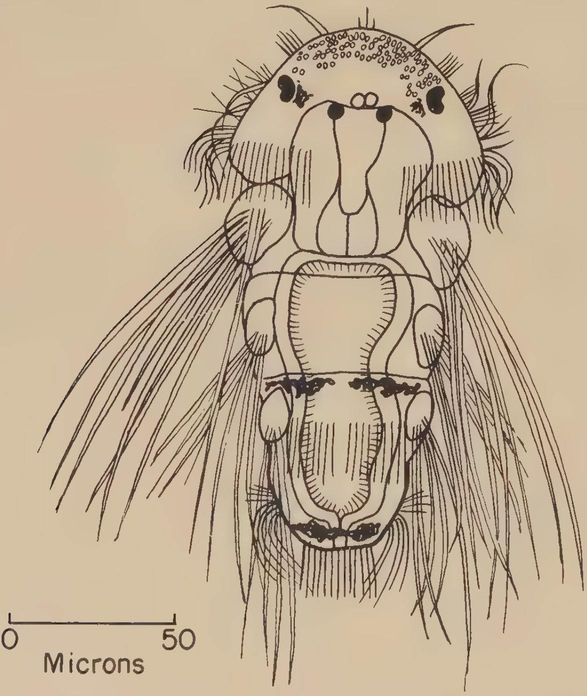

FIGURE 383.—Drawing of newly emerged larva of *P. websteri* viewed alive from the dorsal side. From Hopkins, 1958.

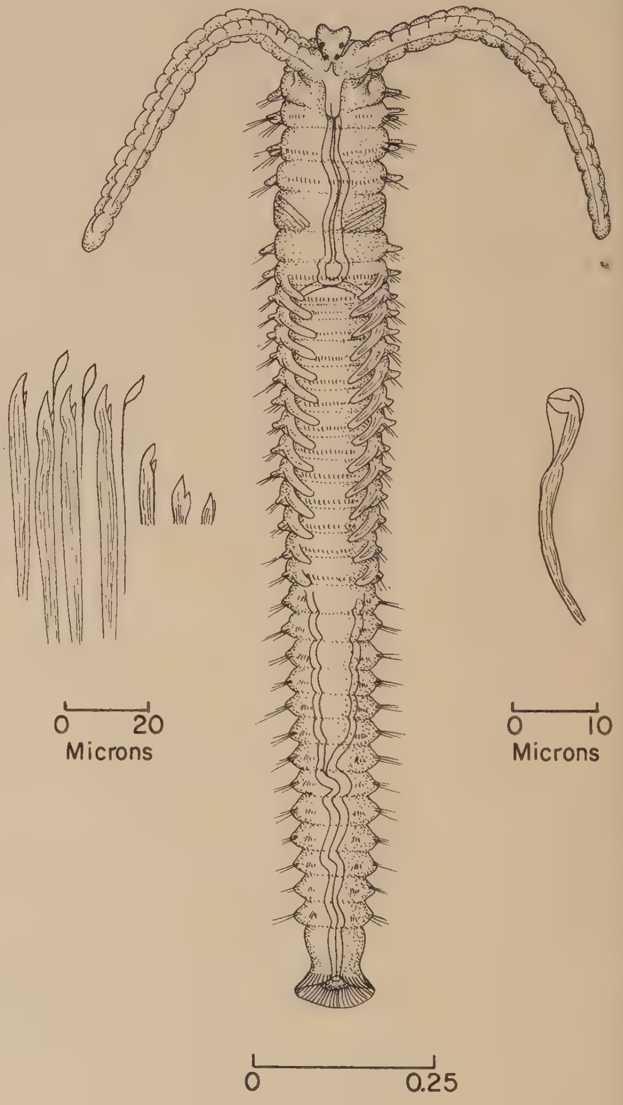

FIGURE 384.—Young "bottom stage" of *P. ligni* Webster. Modified bristles from fifth segment at left, and ventral hooded crochet at right. From Fauvel, 1927.

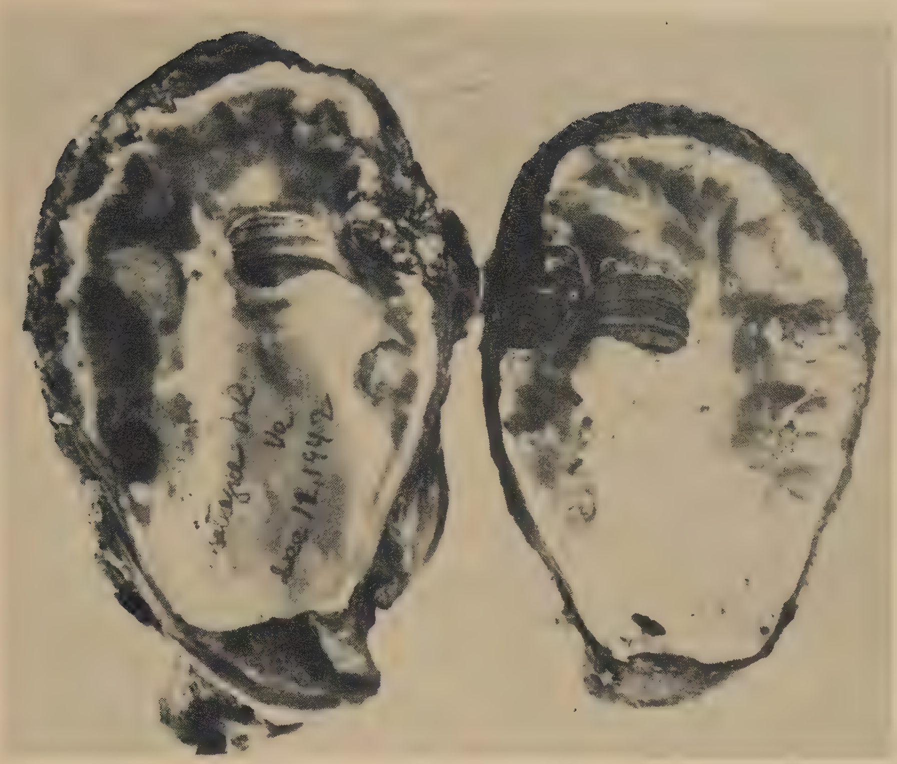

FIGURE 385.—Oyster shells with mud blisters made by *P. websteri* from the grounds at Assateague Island, Va.

evidence, however, that infestation by the mud worm constitutes a serious menace to the oyster population.

Oyster Crab

Several species of the large family Pinnotheridae, commonly called oyster or pea crabs, are associated with oysters, mussels, and other bivalves. The adult females have been known since ancient times and were first described by Aristotle. The males of the American species, *Pinnotheres ostreum* Say, are much smaller than the females and are rarely seen. Usually one or two adult crabs per oyster can be found, and the percentage of infestation varies from zero in some New England waters to about 77 percent in New Jersey. The latter figure, quoted from Christensen and McDermott (1958), refers to the "invasion" of the oyster crab on certain grounds of Delaware Bay. The oyster crab is also abundant in Virginia waters, where its life history has been studied by Sandoz and Hopkins (1947). Some oysters contain a surprisingly large number of these crabs; the maximum reported in a seed oyster was 262 (Stauber, 1945).

Larvae of the oyster crab are pelagic until late summer. At this time larval development is completed, the first crab stage is reached, and the small crabs invade the mantle cavities of oysters. At this time the carapace width of the young crabs ranges from 0.59 to 0.73 mm.

The female crab may be found in various parts of the water-conducting system of the oyster, but settles chiefly on the surface of the gills, in the promyal and suprabranchial chambers, and grows with the growth of the host. The males are not permanently attached to their host and may leave to enter other oysters for copulation.

For many years the oyster crab has been considered an innocuous commensal; however, the female crabs which have settled on the oyster erode its gills and impair their function. More serious lesions may develop and cause leakage of water from the water tubes, which further reduces the efficiency of the food collecting apparatus and of the gills. Rapid regeneration of the damaged gills probably saves many oysters from death, but interference with the normal gill functions causes a relatively poor condition in many infested oysters.

Spirochaetes

Tissues of oysters are often infected by spirochaetes which may be found in the stomach, crystalline style sac and in the gonads after spawning. Dimitroff (1926) identified 10 species and found that 91 percent of the oysters sold in Baltimore, Md. were infected. He reported the following species: *Saprospira grandis* Gross; *S. lepta, S. puncta; Cristispira balbiani* (Certes); *C. anodontae* Keysselitz; *C. spiculifera* Schellack; *C. modiola* Schellack; *C. mina; C. tena;* and *Spirillum ostrae* Noguchi. The species are harmless to oysters and man.

Perforating algae

The empty shells of oysters and other mollusks found on tidal flats and on the bottom are frequently perforated by various algae. Bornet and Flahault (1889) gave a detailed description and illustrations of several species, some of them also found in the carapaces of crabs. Live mollusks do not escape the attacks of perforating algae. *O. edulis* of various ages living in the channel of Saline de Cagliari, Italy, were found to be infested by three species: *Hyella caespitosa* Bornet and Flahault; *Mastigocoleus testarum* Lagerheim; and *Gomontia polyrrhiza* (Lagerheim) (Agostini, 1929). The algae penetrate the periostracum, then spread across the prismatic layer, and form branching threads in the inner layer of shell. Apparently the growing tips of the filaments dissolve the calcium carbonate of the shell and make possible the expansion of algae which, in severe cases of infestation, spread through the entire valve and become noticeable by the greenish color of the valve's inner surface. The color cannot be rubbed off the surface since the alga is separated from the oyster and does not come in direct contact with its body. The algal filaments can be studied on fragments of shell or after decalcification in acid.

Gomontia polyrrhiza, continuously distributed along the Atlantic coast, has been reported from North Carolina and Connecticut, to New Brunswick, Canada, growing in empty shells along the shores and occasionally found in live *Spirorbis* and barnacles (Taylor, 1937).

Live oysters infested with perforating algae are occasionally found in shallow bays and estuaries of Cape Cod. The inner surfaces of the valves are bluish-green. At Woods Hole I saw under a microscope a network of perforating algae resembling *Gomontia* and probably mixed with other species. The plants have not been positively identified.

Perforating algae do not appear to be harmful to oysters. Continuous growth in empty shells accelerates the disintegration of the shells and the return of calcium salts to the sea.

Fouling organisms

Many sedentary marine organisms use oyster shells as a convenient place to attach, either permanently or temporarily. They do not penetrate the shell nor do they inflict any direct injury on the oyster, but they do compete with it for food and space and sometimes smother the oyster by their accumulated mass. The most conspicuous among them is the American species of slipper shell, *Crepidula fornicata* (L.), which received international notoriety because of the havoc it caused for oyster growers in Europe.

Various species of *Crepidula* are very common gastropods found attached to hard objects near or below low water. *C. fornicata* does not present a problem to oyster growers in the United States, although sometimes in certain estuaries, as in Cotuit Bay, Mass., it becomes a nuisance because of its extraordinary abundance. Slipper shells settle on oyster shells and tend to form a spirally curved chain of individuals, the sexes of which change from female to male (fig. 386).

The lowest and, therefore, the oldest members of the chain are always females. The uppermost are males, and those between the two extremes are hermaphrodites, which undergo changes from female to male. To the biologist the species is of interest because the alteration of sex which takes place in this mollusk offers an excellent opportunity for experimentation. Grounds heavily infested with *Crepidula* are, therefore, of great value as a source of material for marine biological laboratories. Oyster growers do not share this enthusiasm because the presence of large numbers of unwanted slipper shells requires additional work in cleaning the oysters before delivery to market.

On many occasions *C. fornicata* has been introduced to Europe with the shipment of live oysters from the United States. It has established itself in Essex, Northumberland, Falmouth, England, and in South Wales. In 1929 the first specimens of *C. fornicata* were noticed in the Oosterschelde, Netherlands, and in 1932 to 1933, according to Korringa (1950), the situation became alarming. The mollusk spread to the German and Dutch

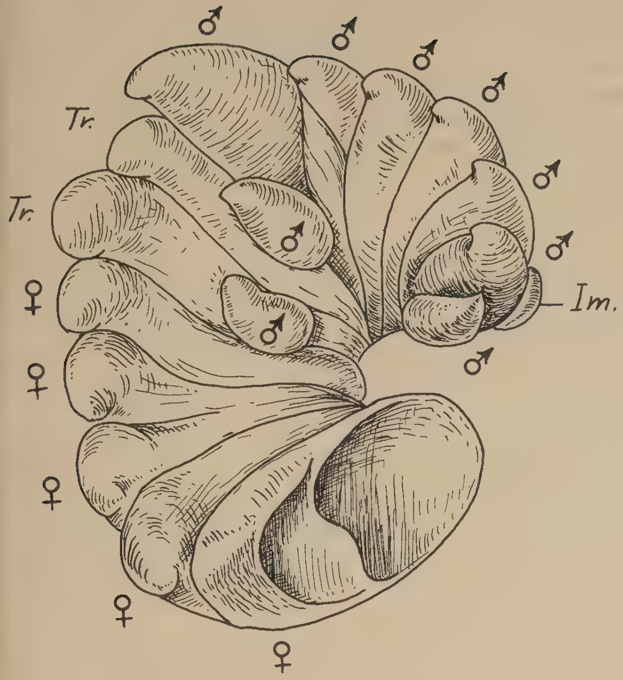

FIGURE 386.—Chain of *C. fornicata*. Female mollusks, the oldest of the group, are at the bottom; the males occupy the uppermost position with the hermaphrodites (tr.) between the two groups. From Coe, 1936.

Wadden Zee and the Limfjord in Denmark, where it successfully competed for space and settled on scattered shells, making it impossible for the larvae of *O. edulis* to set on them. Furthermore, a large amount of silt and soft mud was deposited by *Crepidula* and rendered the bottom unsuitable for oyster planting.

The story of *C. fornicata* is an excellent illustration of the possible danger of introducing a foreign species, which under new conditions, and in the absence of natural enemies and diseases, may reproduce and survive at a rate which upsets the natural balance of nature.

Other fouling organisms may be of a seasonal nature. Some of the oyster bottoms along the Atlantic coast of the United States are often covered with millions of tunicates of the species *Molgula manhattensis* (De Kay). This ascidian can be so abundant in a dredged sample that the oysters are hidden under the gray mass of tunicates. I observed this condition in the mouth of Chester River, Md.; undoubtedly it occurs in other places along the coast. The fouling by *Molgula* is seasonal; the organism dies and the remnants are sloughed off in the fall. Among the 29 species of invertebrates collected from oysters suspended from a raft in the water of Oyster River, Mass., four species constituted the largest portion of the biomass: *Molgula manhattensis*, *Botryllus schlosseri*, *Amphitrite ornata*, and *Balanus balanoides*. The worm *Amphitrite* was found in typical tubes of mud about one-quarter-inch or more in diameter.

At the height of the fouling season in August, the weight of the animals and plants and of sediment accumulated by them comprised 44 percent of the total weight of a string of oysters. The death of *Molgula* in October and the sloughing off of its cases reduced the weight to 11 percent. Later on in November the weight increased to about 17 percent because of the growth of the remaining organisms.

The shells of living oysters are frequently covered with encrusting Bryozoa. In New England waters and in Chesapeake Bay the appearance of Bryozoa usually precedes the time of setting of oyster larvae. When the oysters complete their development, the shell surfaces may be covered with Bryozoa colonies and unsuitable to receive the set of spat. There is a possibility, not fully substantiated, that a great many oyster larvae are eaten by Bryozoa, and Osburn (1932) thinks they are detrimental to the oyster beds in Chesapeake Bay. Marie Lambert made a faunistic study of the Bryozoa collected during the summer on live oysters in the Oyster River near Chatham, Mass. She recorded two species of Endoprocta and five species of Ectoprocta. The most common on oyster shells were *Bowerbankia imbricata* and *Schizoporella unicornis*. The latter is an encrusting bryozoan (fig. 387) commonly found on oyster grounds of Connecticut, part of Long Island Sound (Hutchins, 1945), and Chesapeake Bay.

Dense setting of barnacles on oyster shells is very common throughout the range of distribution of *C. virginica*. In many instances, the space that would have otherwise been available to oyster larvae is already occupied by barnacles, or the spat becomes covered with barnacles and fails to grow. Barnacles have no adverse effect on adult oysters.

The assemblage of invertebrate species found living in close association with the oyster reflects the fauna of the region and naturally differs from place to place. In some areas oysters may be almost entirely free of fouling organisms, while in others their shells are hidden under a heavy mass of siliceous sponges, hydroids, compound ascidians (*Botryllus*), and Bryozoa.

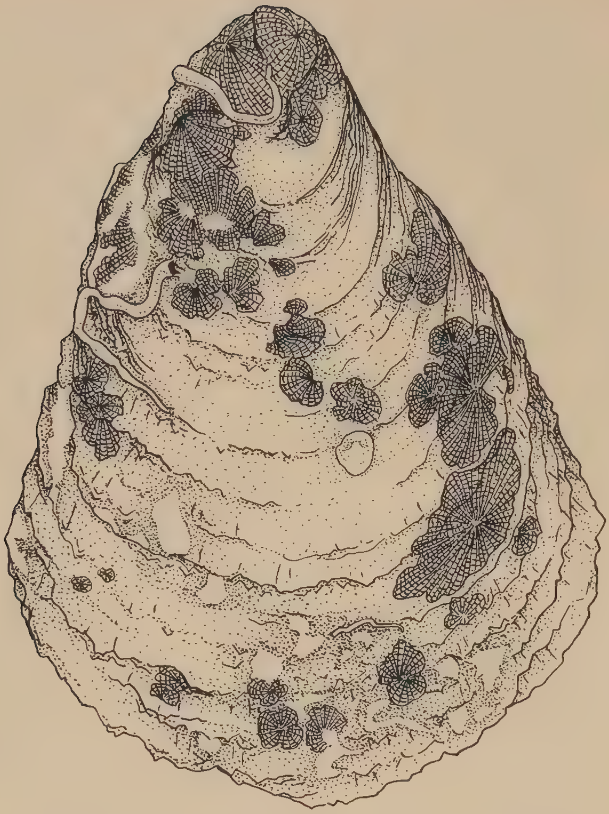

FIGURE 387.—Oyster from Oyster River, Chatham, Mass., covered in part by the colonies of the bryozoan *Schizoporella unicornis* and the compound ascidian (white spots) *Amaroucium constellatum*. Fouling is beginning; within a few days the surface of the shell may be completely covered with these two animals.

The fouling is always seasonal, and with the onset of cold weather many animals and plants die and slough off. Possibly because of the periodicity of fouling, the oysters survive and with few exceptions are not affected by the organisms growing on their shells. An exception is the invasion of oyster beds by mussels (*Mytilus edulis* L.), which in several situations may completely cover an oyster bed with a thick layer of mud mixed with excreta.

A number of annelids are commonly associated with oyster communities, living between clusters of oysters or in the shells. Sometimes a surprisingly large number of worms crawl out of the shell crevices when Epsom salt is added to the water in which oysters were kept. Hartman (1945) lists seven species of annelids inhabiting the spaces between clusters of living oysters. Korringa (1951b) describes more than 30 species of annelids which in Dutch waters live on or in the shell of *O. edulis*. Except for the boring Spionidae, the worms apparently cause no direct harm to oysters, but some of the mud-gathering species of Nereidae materially increase the deposition of sediment over an oyster bed. No evidence has been found of any other adverse effects of annelids on oyster communities.

Various siliceous sponges are very common members of the epifauna of oyster bottoms. With the exception of the boring sponges, they do not affect oyster populations. The red sponge, *Microciona prolifica*, is often found on highly productive oyster bottoms.

Of the protozoa that live on oyster shells, the stentorlike infusorian, *Folliculina* sp., commonly inhabits brackish water beds. This relatively large protozoan, measuring from 200 to 800 μ, lives in bottle-shaped cases attached to the leaves of *Elodea*, and *Potamogeton* found in the mouths of rivers and on shells of other mollusks. During the warm season it rapidly multiplies and appears swimming with other plankton. Mass occurrences of folliculinids in the Chesapeake Bay were recorded by Andrews (1915) and in Oosterschelde, Netherlands, by Korringa (1951a). Different species are widely distributed in the coastal waters of the United States (Andrews, 1944). The number of this infusoria found attached to a single oyster shell has varied from one to several hundred.

In many localities along the eastern shore of the United States, oyster beds are frequently overgrown by various algae. *Gracillaria confervoides* (Linnaeus) Greville is one of the species which sometimes completely covers an oyster bottom with its thick growth. Huge masses of the plant wash away from the home grounds and pile on beaches. Of the many other algae found growing on oyster shells, several are in some regions as abundant as *Gracillaria: Enteromorpha, Ulva, Griffitsia, Ceramium, Chondria, Champia,* and *Scytosiphon*. During experiments on raft culture in Oyster River near Chatham, Mass. in 1956 to 1959 (Shaw, 1962), the shells of young oysters suspended in water became covered with a very dense growth of *G. confervoides*. Since there was no noticeable ill effect on the oysters, an examination was made of the periphyton, the organisms living loosely attached to the plant's branches. The prevailing form was found to be a diatom *Lycosoma* sp., which was not present in the river plankton outside the immediate area occupied by *Gracillaria*. The stomach content of the oysters consisted of many *Lycosoma*, some half-digested, and of *Skeletonema*, which was also abundant among the branches. It is apparent that some constituents of the periphyton may be ingested and that the microscopic flora of the environment provides a substantial amount of food not available in true phytoplankton.

Some of the seaweeds cause unexpected damage to commercial oyster grounds. *Colpomenia sinuosa* (Toth) Derbes and Solier, a common seaweed in many parts of the word, is one of them. It grows along the Pacific Coast of North America from Alaska to southern California, along the eastern coast of Australia and in France. The thallus of *Colpomenia* is of a papery texture and hollow; it can grow attached to oyster shells to the size of a hen's egg or tennis ball. On sunny days at low tide in shallow water photosynthesis may be so intense that gas bubbles fill up the thallus, and on the return of the tide the inflated balloon floats out to sea carrying with it the young oysters. In 1906 *Colpomenia* became such a nuisance on the western coast of France at Vannes that the oystermen called it "oyster thief." The floating oysters carried out by the ebb current were not returned to shore with flood tide, and the losses were severe enough for local oystermen to organize the recapture of oysters with nets and to tear off the inflated algal ballons by dragging faggots over the bottom (Church, 1919).

In 1961 the seaweed, *Codium fragile* subsp. *tomentosoides* (Goor) Silva, was introduced to Cape Cod waters with oysters brought from Peconic Bay, Long Island, N.Y. This Pacific Ocean species, not indigenous to Massachusetts, occurs in abundance along the western coast of Europe. It is not known how the alga was introduced to Long Island where in January 1957 it was found at East Marion attached to dead *Crepidula* shells (Bouck and Morgan, 1957). In Oyster River, near Chatham, Mass., where the Long Island oysters were planted the shells were covered with a luxuriant growth and had to be thoroughly scrubbed before being shipped to market. The following year the plants were so large (fig. 388) that on sunny days they acted as "oyster thieves" by lifting the oysters from the bottom with gas-filled branches and floating them off with the tide.

Eel grass, *Zostera marina*, frequently covers the

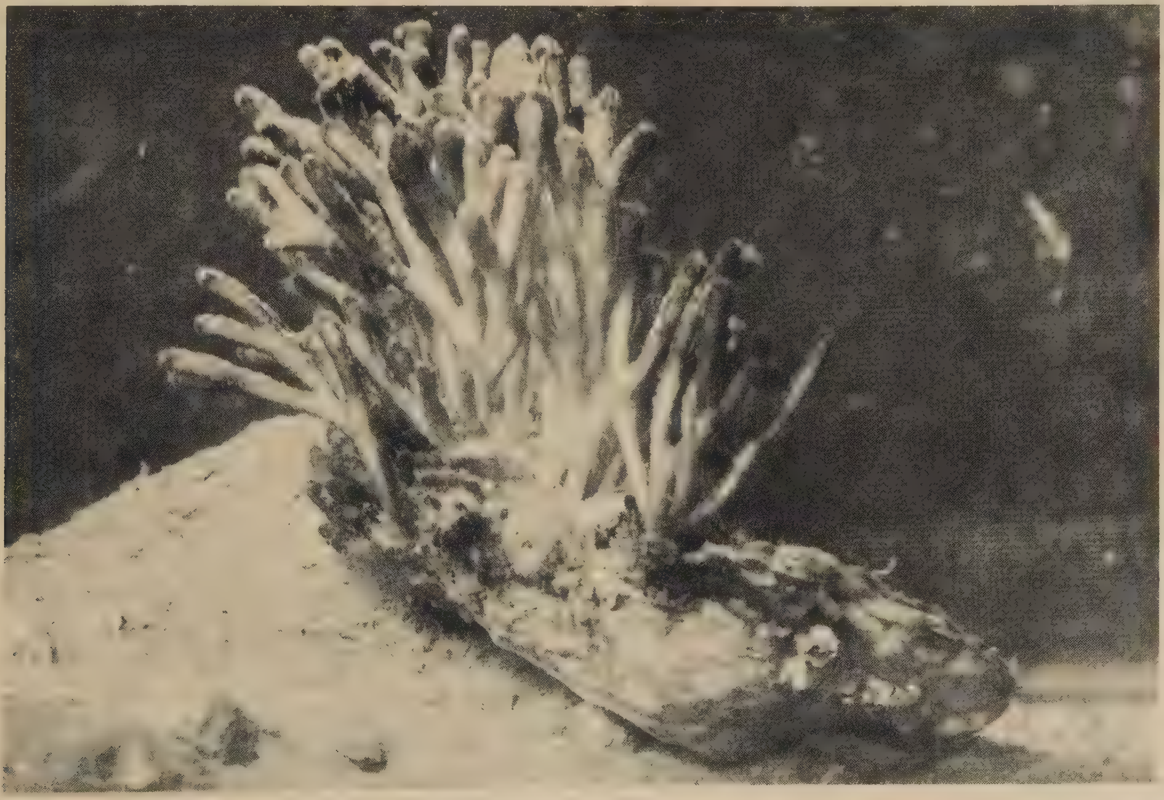

FIGURE 388.—*C. fragile* introduced into Oyster River, Chatham, Mass., with oysters from Long Island. Two-year-old plant.

entire oyster bottom but apparently exerts no ill effect on oyster populations. This is not the case, however, with another aquatic plant, the Eurasian watermilfoil, *Myriophyllum spicatum*, which by 1933 was established on the Virginia and Maryland sides of the Potomac River; since 1959 it has increased rapidly in the Chesapeake Bay area, including the Potomac River, and is found also in the fresh and nontidal waters above Washington D.C. In recent years the growth of the plant has become spectacular and is a threat to brackish water oyster grounds, which may become covered with a heavy layer of decomposing leaves and stems of the milfoil (Beaven, 1960; Springer, Beaven, and Stotts, 1961).

The effect of commensals and competitors on the productivity of an oyster bottom can be evaluated for each species if the intimate relationship to the host is clearly understood and the relative abundance of a species is determined. A single species which appears to be innocuous under normal conditions may become destructive and dangerous because of its mass development. All these conditions should be evaluated in order to express their effect in numerical terms. Commensals such as bryozoans, barnacles, and tunicates so completely cover the shell surface that the settlement of young oysters upon it is prevented. Thus the negative effect of fouling may be considered in relation to the productivity of setting grounds. On the other hand, in southern waters where setting continues for the greater part of the year and oysters become overcrowded with successive generations of young, the reduction and prevention of settlement of spat may be beneficial because it reduces overcrowding and permits better growth and fattening of oyster stock. The struggle for space is an essential factor in the life of an oyster community.

PREDATORS

The list of many enemies that prey on oysters includes flatworms, mollusks, echinoderms, crustaceans, fishes, birds, and mammals. Not all of them are equally destructive to oyster populations. The most dangerous are those which prefer oyster meat to other types of food, and in search of it invade the oyster grounds.

Carnivorous gastropods

The deadliest enemies of oysters are various gastropods inhabiting coastal waters. The most widely distributed species is the common oyster drill, *Urosalpinx cinerea* (Say), which is found along the entire Atlantic Coast from Canada to Florida. With a shipment of *C. virginica* the common oyster drill was introduced to the Pacific Coast of the United States (1870 and the following decades), and to Great Britain (1920 and probably earlier) where the American oysters were planted at Brightlingsea and West Mersea. In a short time the drill became very abundant along the coast of Essex and across the Thames estuary. At present *Urosalpinx* is the most dangerous and the most widely distributed of all the predators of oysters in Europe.

Oyster planting by shellfish growers is the major factor in the wide dispersal of *Urosalpinx* in this country and its introduction into areas which formerly were free of the pest. The migration of drills is rather limited. When hungry, they may move at an average rate of 15 to 24 feet per day in the direction of food. To a certain extent the drills are dispersed by floating objects to which they may cling and by hermit and horseshoe crabs which have been seen bearing as many as 140 drills per animal (Carriker, 1955).

The drill is particularly destructive to young oysters. In Cape Cod coastal waters, which are infested by these snails, the oyster spat has very little chance of surviving the first year; often small seed oysters are wiped out before the end of the second year. Adult oysters with thick shells suffer less, and the losses sustained during 1 year by the 4 and 5 year classes are insignificant. There are many localities in Long Island Sound, on the eastern shore of Virginia between Chincoteague and Cape Charles, and in other regions where drills commonly kill 60 to 70 percent of the seed oysters and sometimes annihilate the entire crop.

Fortunately, brackish water effectively bars the drills from the upper parts of estuaries and tidal rivers. Survival of drills in water of low salinity depends on temperature and on the concentration of salts to which they were adjusted. It may be accepted as a general approximation that minimum survival salinities at summer temperatures vary from 12°/₀₀ to 17°/₀₀ in different regions.

Extensive literature on the biology and control of oyster drills has been critically reviewed by Carriker (1955), who has also made a detailed study of the structure and function of the proboscis and drilling apparatus of the drills (Carriker, 1943).

The maximum height of adult drills varies in different localities between 25 and 29 mm.; a giant form reaching 51.5 mm. in height is found in the area of Chincoteague Island, Va., and is considered a subspecies *U. cinerea follyensis* (fig. 389). As the common name indicates, the *Urosalpinx* attacks oysters and other mollusks by drilling a round hole in the shell. The hole, usually made in the upper (right) valve of the oyster, tapers toward the inner surface; the shape of the hole identifies the attacker, and the presence of drilled empty shells on oyster grounds is reliable evidence of the inroads made by the snail on an oyster population.

For a long time boring was considered an entirely mechanical process. Observations made by Carriker (1961a) showed that both chemical and mechanical actions are involved. Secretion from the accessory boring organ, called ABO for short, softens the shell, probably by an enzyme acting on the conchiolin, and the softened material of the shell is removed by abrasive action of the radula. Active drilling continues for a few minutes and is followed by a long period lasting up to an hour of chemical action during which the ABO gland remains in contact with the shell.

The oyster is not the only victim of drills. They show preference, in fact, to barnacles, and usually stop drilling oysters if a rock covered with live barnacles is placed near by. A well-developed chemical sense permits the drills to distinguish between young and adult oysters. If both kinds are offered to hungry snails kept in a large tank with running sea water, the majority of active drills will choose the young oysters. The drills are positively rheotactic and in running water orient themselves against the current. The orientation is not, however, precise and the path of a moving drill is a meandering line only generally directed against the current.

Light has an effect on the orientation of drills. They move away from a strong source of light, but move toward it at lower intensities (Carriker, 1955). In dim light, the phototactic response is lost. In laboratory tests at Woods Hole, I noticed no orientation of drills toward the window side of the tank; the drills distributed themselves at random. They have a tendency to climb away from the bottom (negative geotaxis) and congregate on rocks, pilings, and on the wall

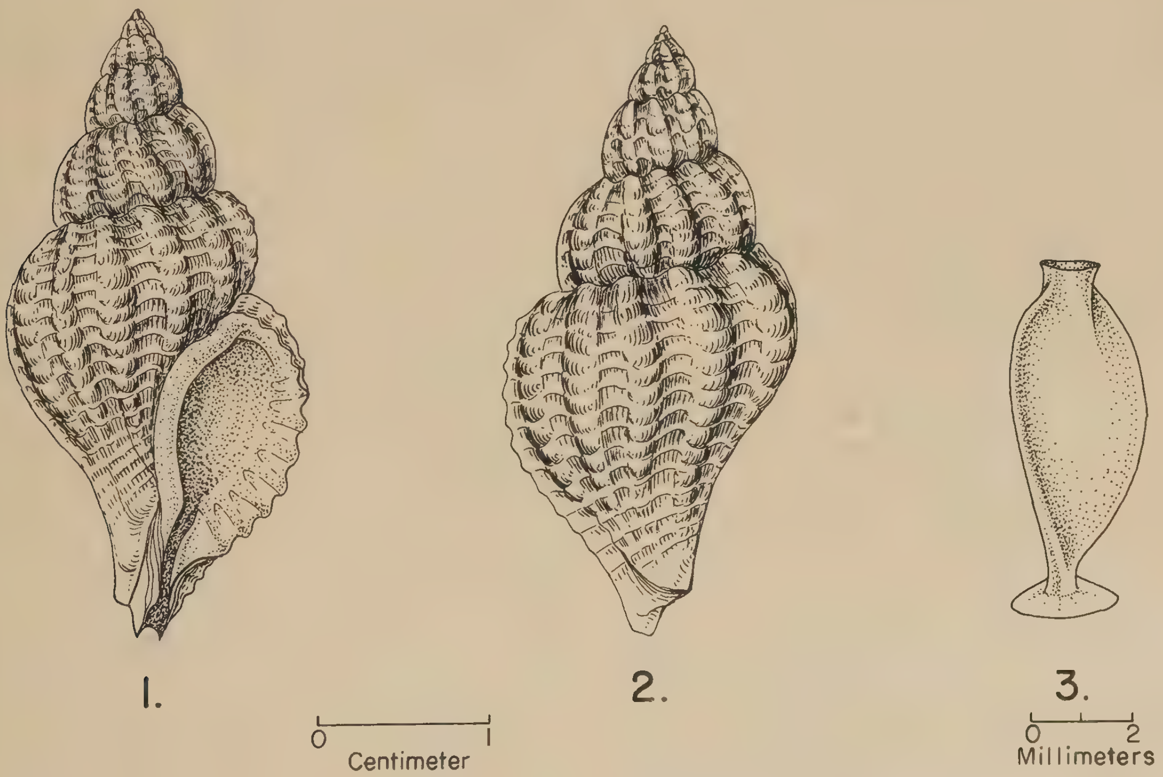

FIGURE 389.—*U. cinerea follyensis* from Chincoteague Island region, Va. 1—apertural view; 2—abapertural view; 3—egg cases.

of tanks. Drills put in the bottom of a vertical glass tube about 1 inch in diameter and 6 to 8 feet tall, filled with sea water, will climb to the top of the tube and remain there. Negative geotaxis is pronounced, particularly during the reproductive period. At this time drills climb on any objects above the bottom level and ascend rocks and various underwater structures to lay their eggs, which are deposited in tough, leathery capsules (fig. 389, 3). The egg-laying period depends on geographical location and local conditions. Summarizing the data from various sources, Carriker (1955) estimates that the number of egg cases deposited by one drill per season ranges from 0 (in an immature female) to 96 for older females. In Woods Hole harbor, the breeding season lasts from the end of June to the middle of August. The number of egg cases deposited by a single female kept in laboratory tanks varied from five to nine; the average number of eggs in each case was nine. The number of eggs per egg case varied in different localities from eastern Canada to Chesapeake Bay from 0 to 22 and from 1 to 29 in British oyster beds. (Cole, 1942).

A second species of drill, *Eupleura caudata* (Say), (fig. 390), is found in the same waters as *Urosalpinx* but is usually less abundant. Various observers estimate that in different locations it comprises from 2 to 29 percent of the total drill population (Carriker, 1955). The behavior of *Eupleura* is similar to that of *Urosalpinx*. Its food habits have not been studied, but occasional observations in the laboratory indicate they are probably not different from those of *Urosalpinx*.

In the York River, Va., where the growth and reproduction of *Eupleura* were studied by MacKenzie (1961), the snail becomes active as the temperature rises over 10° C. Spawning begins late in May at 18° to 20° C., reaches a peak in June and early July at 21° to 26° C., and ends in early August. Mature females (kept in cages) deposit an average of 55 cases, each containing an average of 14 eggs. In the absence of mortality, each female *Eupleura* may produce over 700 young drills each season. The leathery egg capsules are vase-shaped with two distal projections (fig. 390, 3) and are easy to distinguish from the egg cases of *Urosalpinx* (fig. 389, 3).

Conchs of the genus *Thais* occur on both the Atlantic and the Pacific coasts. The snails have strong polymorphic tendencies and form local races which greatly complicate the taxonomy of the species. A review of the speciation problem of *T. lamellosa* Gmelin made by Kincaid (1957)

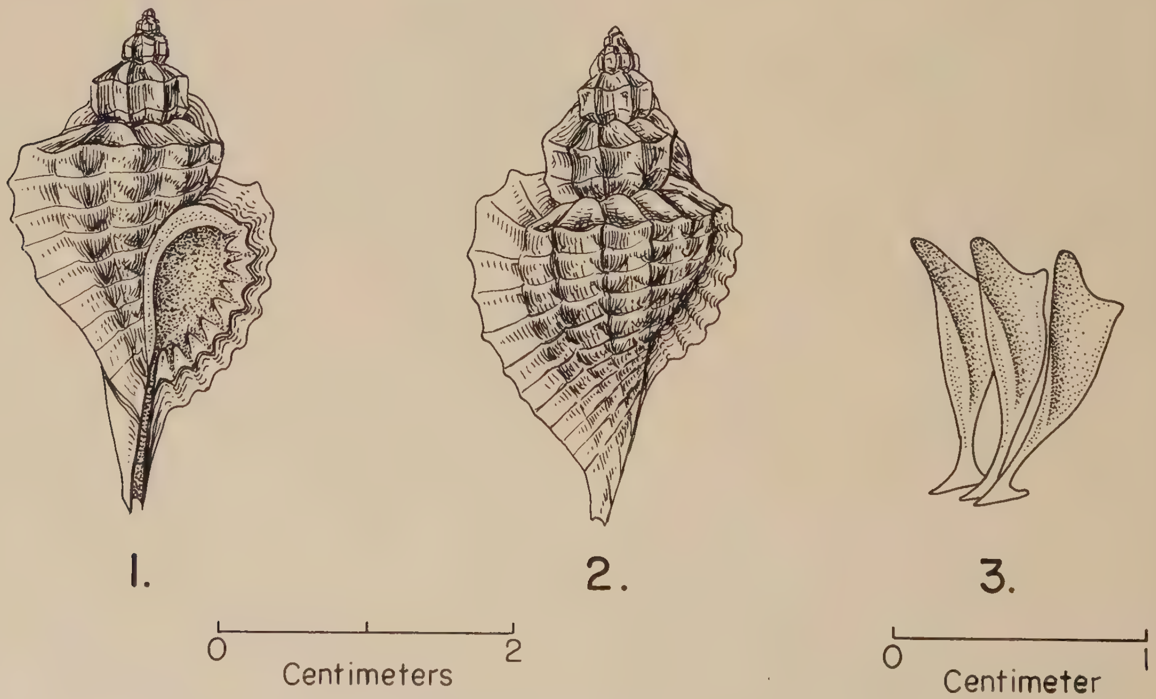

Figure 390.—*Eupleura caudata* (Say) from 1—apertural side; 2—from abapertural side, 3—egg cases.

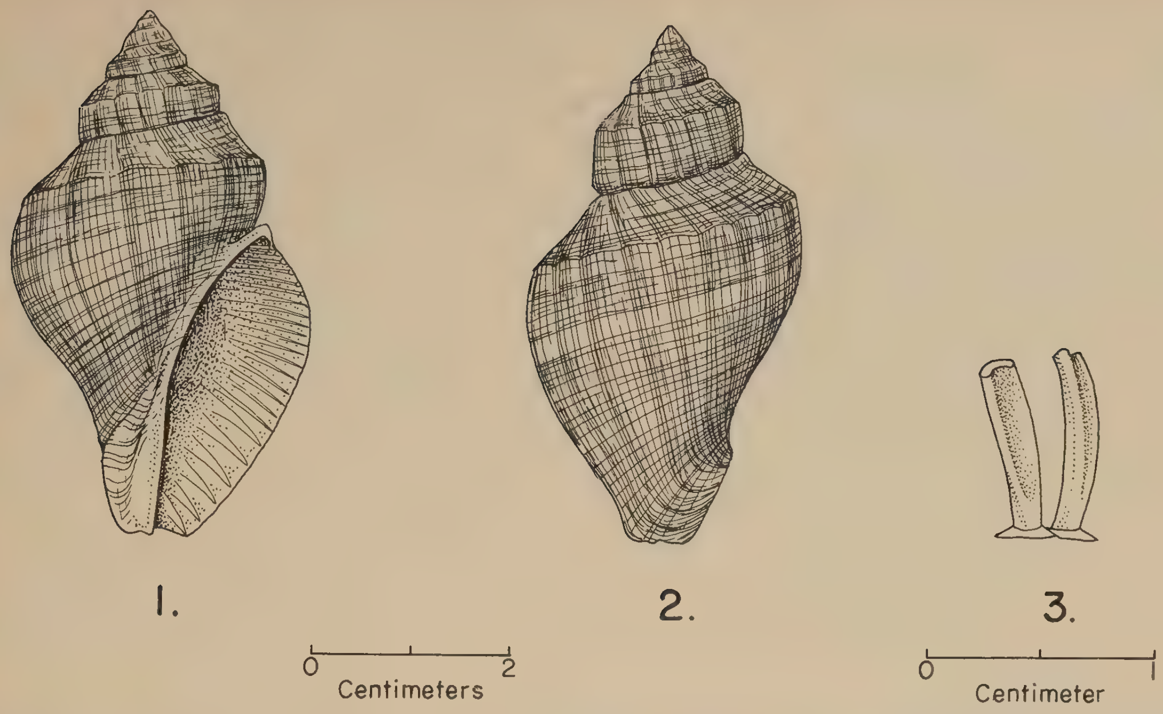

FIGURE 391.—*T. haemastoma floridana* Conrad from the shores of Pensacola Bay, Fla. 1—apertural view; 2—abapertural view; 3—egg cases.

contains interesting material regarding this and other species of the genus.

T. haemastoma is a common oyster predator in the waters of the South Atlantic and Gulf states. There are two subspecies, *T. haemastoma floridana* Conrad, which occurs from North Carolina to Florida and the Caribbean (fig. 391), and *T. haemastoma haysae* Clench (fig. 392), common on oyster grounds of northwest Florida, Louisiana, and Texas (Clench, 1947). *T. haemastoma floridana* is a medium-size gastropod with a relatively smooth shell and a single row of low spines. *T. haemastoma haysae* is a large, rugged snail, sometimes measuring 4½ inches in height. It can be distinguished from the other subspecies by double rows of prominent spines around the whorls and spire.

The behavior of the two varieties apparently is similar. The conchs feed on oysters and other mollusks, penetrating their shells from the edge by using the ABO gland or by drilling holes in the shell (Burkenroad, 1931; Carriker, 1961a). The entrance at the edge of the valves is often inconspicuous and may be easily overlooked.

Conchs multiply very rapidly because of their great fecundity and high survival rate of larvae. *Thais haemastoma* lays eggs in groups of about 800 to 975 enclosed in each egg case, with each female depositing more than 100 cases. These figures refer to my laboratory observations on conchs kept in captivity. The eggs are deposited in horny and transparent egg cases of a creamy color, which becomes brownish and finally turns reddish-purple. The breeding season in Louisiana waters begins by the end of March and reaches its peak in April and May. There is usually a rapid decline of egg laying in June and a complete cessation of reproduction in July. At the beginning of the breeding period the conchs become very active and develop a strong tendency to climb on structures and rocks to attach their egg cases above the bottom. Because of this behavior they can be trapped during the breeding season on stakes which the oyster growers erect on the grounds. Gregariousness is very pronounced, and many conchs can be trapped in this way in a relatively short time. The number of egg cases attached to a single stake may be enormous. One stake which I obtained as a sample was covered with a solid mass of egg capsules over a 5-foot length; the estimated number of cases was about 8,000. The incubation period is not known definitely, but judging from the growth of hydroids and other fouling animals on the conch cases, I believe it is not less than 2 weeks.

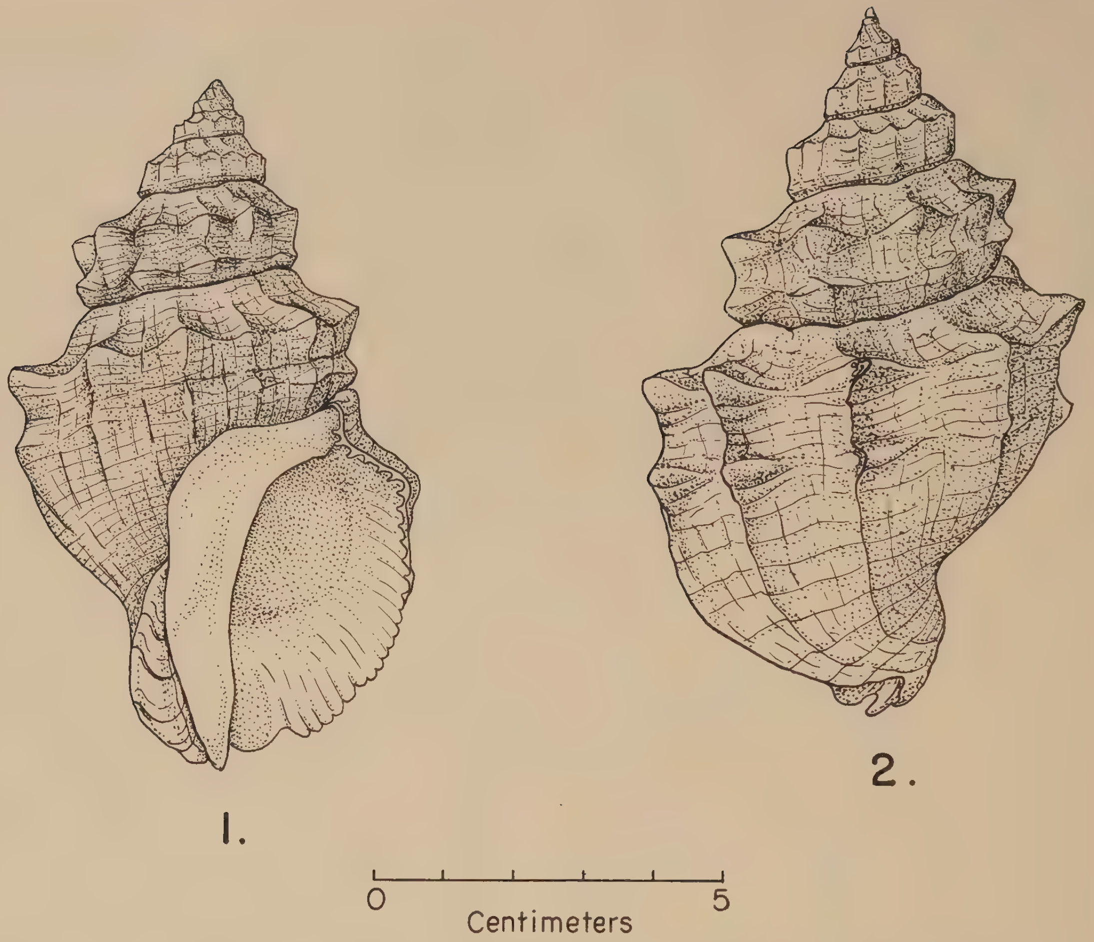

FIGURE 392.—*T. haemastoma haysae* Clench from Bastian Bay in the lower part of the Mississippi River delta. 1—apertural view; 2—abapertural view. Natural size.

The larvae that escape from the egg capsules are veligers, which pass through a free-swimming period of unknown duration, and are widely dispersed by tidal currents before they settle on the bottom and begin attacking small oysters and other bivalves.

The distribution of *Thais* is checked by fresh water. The conch is immobilized by a salinity of 10‰, and a 1- or 2-week exposure to a salinity of 7‰ kills them (Schechter, 1943).

The effect of sudden changes in water salinity on the rate of crawling of *Thais* was corroborated by my observations at the Bureau of Commercial Fisheries Biological Laboratory at Gulf Breeze in northwestern Florida. The crawling of these snails in the tanks was automatically recorded on a kymograph. The movements stopped immediately when the salinity of the water was artificially reduced from 15‰ or 17‰ to 8‰ or 9‰. The snails became active again when the salinity returned to the former level.

Two species of conchs found on oyster grounds of the Pacific coast are *Thais lamellosa* Gmelin (fig. 393), a native snail, and *Ocenebra* (*Tritonalia*) *Japonica* Dunker (fig. 394), introduced from Japan. *T. lamellosa* has been considered by some fishery biologists as a predator on *O. lurida,* but Kincaid (1957) discards this view as not substantiated by his 50 years of familiarity with the marine fauna of the region. He states that since *T. lamellosa* feeds mainly upon barnacles and mussels, the snail should be classified as "the only invertebrate friend" of the oyster, presumably because it destroys its competitors. Chapman and Banner (1949) found that under experimental conditions *T. lamellosa* drilled some *O. lurida,* but that in a natural environment it showed a preference for mussels (*Mytilus edulis*).

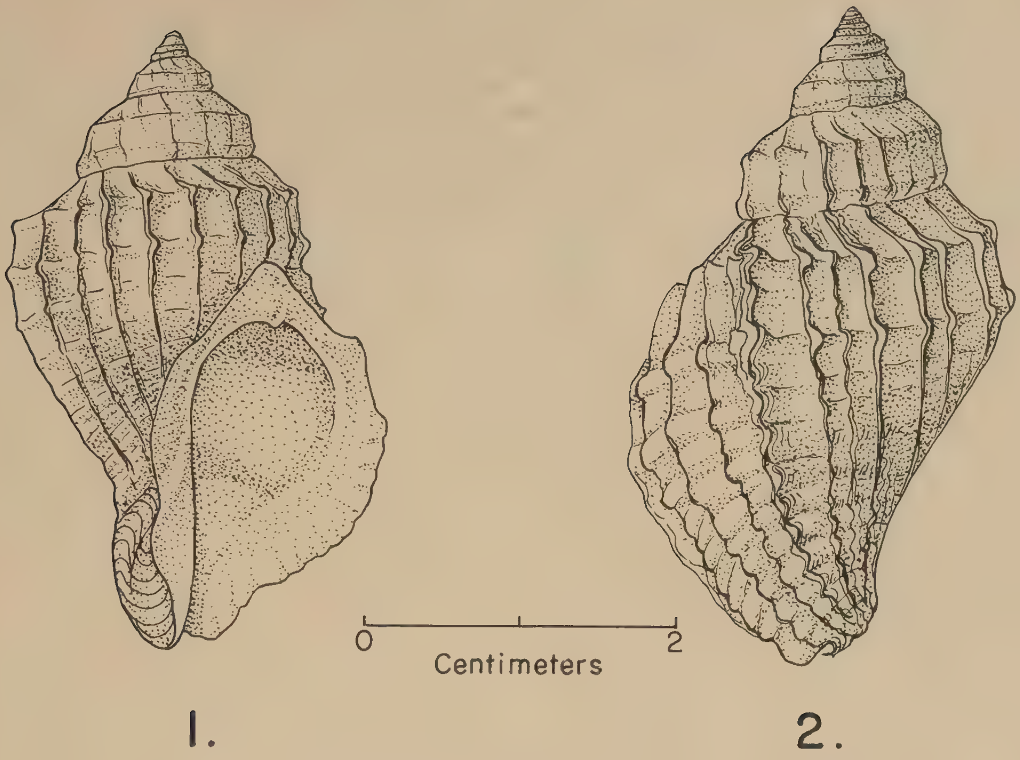

Figure 393.—*T. lamellosa* Gmelin, a native species of the Pacific coast of the United States. 1—apertural view; 2—abapertural view.

The Japanese species, *Ocenebra japonica*, is far more dangerous than the native snail. Mortalities due to devastation by this snail are estimated at 15.4 to 22.6 percent. The first specimens of *O. japonica* were introduced into the waters of Puget Sound with the planting of Japanese seed oysters, a practice which began in 1902 and 1903 and which reached considerable proportions by 1922 when from 1,500 to 4,000 boxes, each containing about 5,000 seed, were planted annually. In October 1928 while examining the oyster beds in Samish Bay, Wash., I found a number of *O. japonica* Dunker (Galtsoff, 1929, 1932), and warned oystermen and state officials of the possible damages that could result if the practice of bringing infested seed oysters from Japan was continued. The warning received no attention. In the late 1940's *Ocenebra* was well established in the waters of Puget Sound and became a serious menace to the native oysters. When given a choice of food, *Ocenebra* prefers *O. lurida* and Manila clams, *Venerupis japonica*, to *C. gigas* (Chew, 1960). It drills holes in the shell by combined chemical and mechanical action (Carriker, 1961a). The fertility of the species is high, the female laying an average of 25 egg cases, each containing about 1,500 eggs. The egg cases are often found in the inaccessible crevices of the concrete walls of dikes surrounding the Olympia oyster beds. Salinity of 18‰ adversely affects *Ocenebra*, and brackish water of less than 12‰ salinity is lethal.

Large conchs or whelks, *Busycon carica* Gmelin and *B. canaliculatum* Linné, are common in the shallow water of the Atlantic coast and occasionally attack oysters and open them by inserting the edge of the shell between the valves and forcing them apart (Colton, 1908). Carriker (1951) reinvestigated the problem and found that penetration of shells of oysters and clams is a purely mechanical process which consists of chipping by the edge of the conch's shell combined with rasping of the radula. The shell edge of an oyster destroyed by these conchs bears the marks of the attack (fig. 395). In the northern part of Cape Cod, *Busycon* seems to attack the oyster in preference to other mollusks, annelids, or dead fish, which they are known to consume. Local depredations on oysters observed in the Cape Cod area (Shaw, 1960, 1962) may be severe enough to warrant trapping of conchs during their reproductive

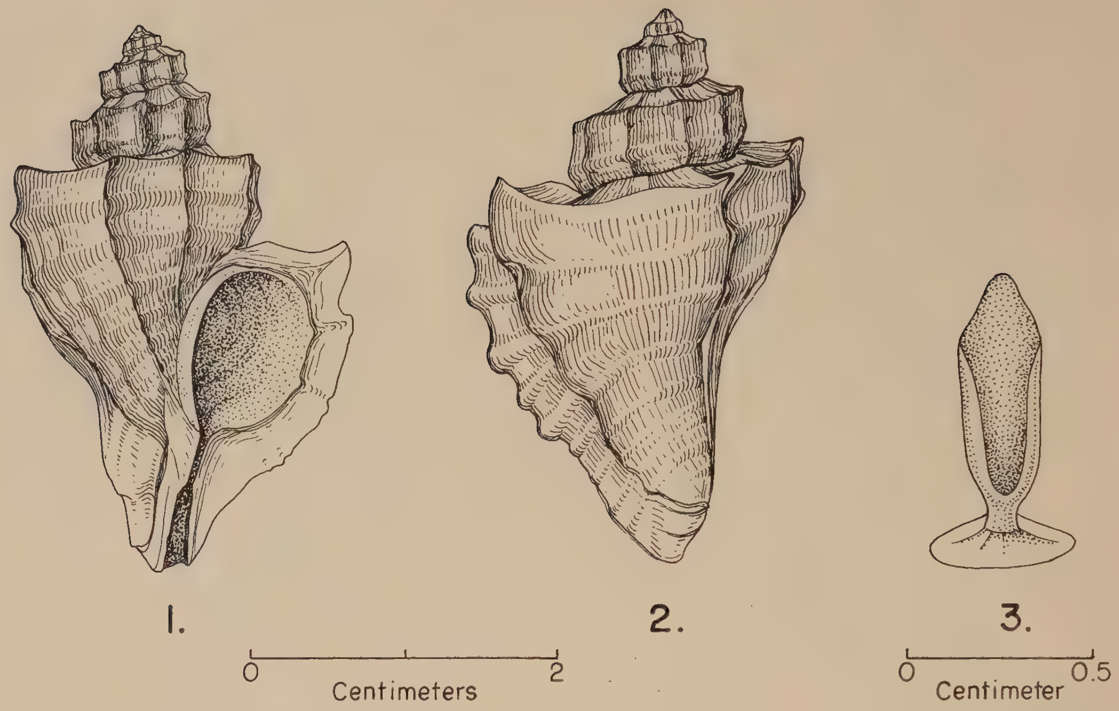

Figure 394.—*O.* (*Tritonalia*) *japonica* Dunker, Japanese species from oyster bottom of Puget Sound, Wash. 1—apertural view; 2—abapertural view; 3—egg case.

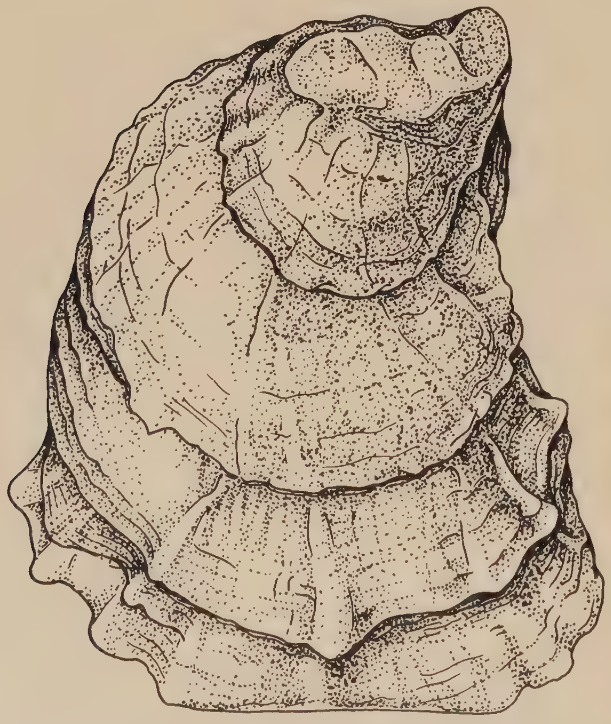

Figure 395.—Edge of the shell of an oyster killed by *Busycon* in Oyster River, Chatham, Mass. Straight line at the lower edge of the shell indicates the place of rasping by the conch's radula after the valves were chipped.

cycles. In Cape Cod estuaries, egg cases of conchs are a familiar sight on tidal flats at low water (fig. 396). Under experimental conditions the conchs were found to consume in summer about three adult oysters per week (Carriker, 1951).

Small parasitizing pyramilid snails of the genus *Odostomia* (*Menestho*) congregate in large numbers at the very edge of oyster shells. When the valves are open, the snails extend their pro-

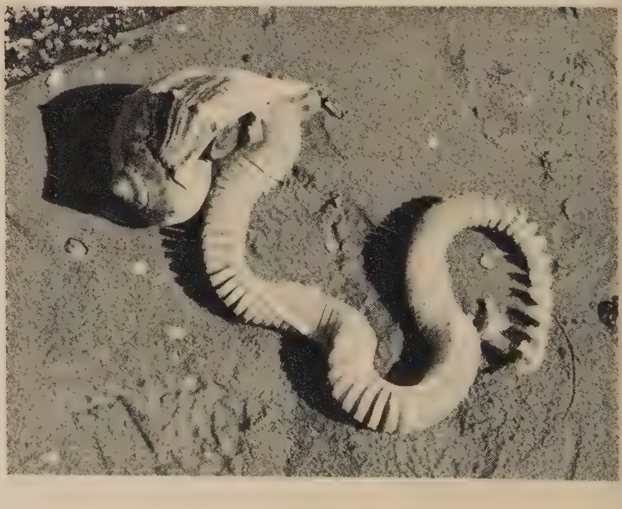

Figure 396.—*B. carica* depositing egg capsules at low Tide. Woods Hole.

boscides to the edge of the oyster's mantle and feed on the mucous and tissues. These ectoparasites are probably a great nuisance to the oyster, but there is no evidence that they can be regarded as important enemies. Two species have been found associated with *C. virginica: O.* (*Menestho*) *bisuturalis* Say which has a range from New England to Delaware Bay, and *O.* (*Menestho*) *impressa* Say which is found from Massachusetts to the Gulf of Mexico.

Starfish

The starfish of the Atlantic Coast is also a highly destructive predator on oysters. The common species, *Asterias forbesi* (Desor), is the most familiar animal in tidal pools, on rocks, and beaches of the Eastern Coast of the United States, often found exposed by the receding tide. Accurate statistics of the destruction caused by this species are not available, but a few selected examples emphasize its deadly efficiency. In 1887 the State of Connecticut estimated the loss caused by starfish at $463,000; the sum represented the destruction of over 634,246 bushels of oysters or nearly half of the total harvest for the year (1,376,000 bushels). The numerical strength of a starfish population over a relatively small area can be visualized from the record of only one company which in 1929 removed over 10 million adult starfish from 11,000 acres of oyster grounds in Narragansett Bay.

As a rule the starfish populations on various parts of the coast fluctuate within wide limits with years of great abundance usually followed by relative scarcity. These fluctuations cause many oystermen to believe that starfishes invade their grounds periodically. Studies of the problem conducted simultaneously in Buzzards Bay, Narragansett Bay, and Long Island Sound (Galtsoff and Loosanoff, 1939) demonstrated that sudden increases in the abundance of *A. forbesi* are due primarily to the high percentage of survival of its free-swimming larvae and their successful setting (fig. 397).

The reproductive season of *A. forbesi* in New England waters slightly precedes that of *C. virginica.* When oyster larvae reach setting stage, the space available for their attachment is already occupied by young starfishes only several mm. in diameter, hungry, and ready to attack the spat. The new set of oysters may be completely wiped out by young starfish.

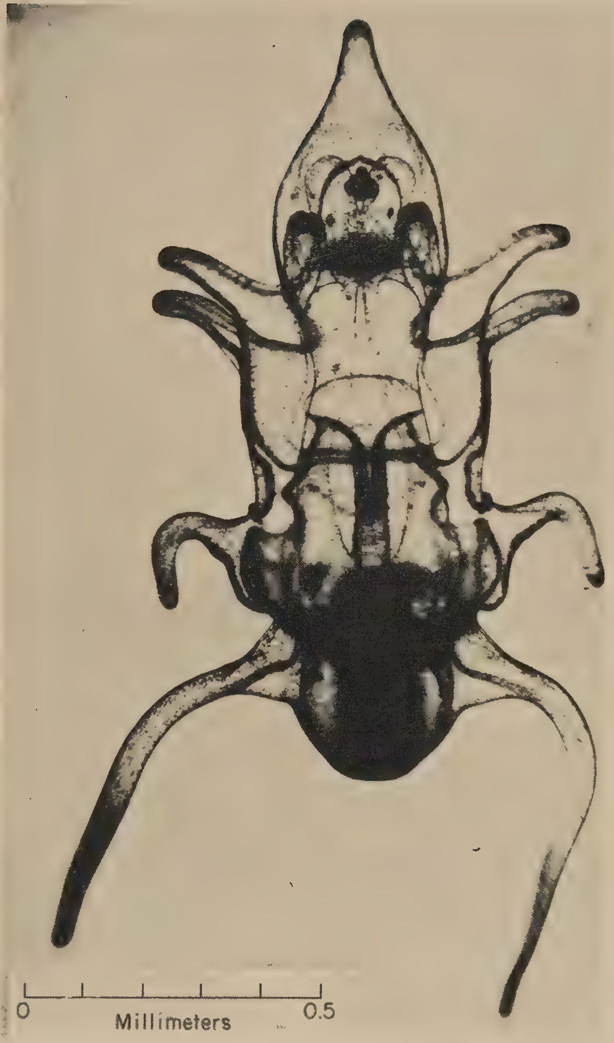

FIGURE 397.—Photomicrograph of live larva, brachiolaria, of *A. forbesi* from a plankton tow in Buzzards Bay.

The movements of *A. forbesi* in concrete tanks are slow, random, and apparently not directed by tactic reactions. Initially it was difficult to reconcile this fact with the experience of oyster growers in Long Island Sound who reported that oyster bottoms thoroughly cleaned by mopping or dredging were invaded within the next 24 hours by swarms of starfish. Underwater observations were made in Long Island Sound by members of the Bureau of Commercial Fisheries Biological Laboratory in Woods Hole who used an underwater television camera. The underwater photographs showed clearly that starfishes are passively transported by the tidal currents which in Long Island Sound are fairly rapid. The animal curls up the tips of the rays, releases its hold on the substratum, and floats just above the bottom.

Many thousands of starfish are transported in this way from place to place and settle on new grounds when tidal currents slacken.

The starfish leaves no identifying marks on its victim, and only empty shells remain as evidence of a destructive attack. The recent death of oysters is indicated by the cleanliness of the valves, which contain no foreign growth and are still attached to each other. The method by which the starfish succeeds in forcing oysters or clams to relax their muscles and open the valves has puzzled biologists for a long time. It seemed doubtful that the starfish could exhaust its victim and open it by main force, and suggestions were made, not well corroborated by observations, that the prey was killed by suffocation or that a substance secreted by the stomach of the starfish produced relaxation of the adductor muscle of the oyster. Sawano and Mitsugi (1932) reported that an extract of starfish stomachs poured over the heart of living molluscs produced tetanus and often inhibited the heart beat; this seemed to give some support to the "anesthetic" hypothesis. Critical experiments made in Woods Hole by Lavoie (1956) show, however, that the effects of extracts prepared from digestive organs of starfish and introduced into the adductor muscle or poured over the heart of *Mytilus* were generally identical with those produced by plain water. On the other hand, the force exerted by the tube feet of starfish in opening shellfish was measured manometrically and was found to exceed 3,000 g. The measurement was made using mussels in which the adductor muscles were severed and replaced by steel springs or plastic cylinders.

Lavoie noticed that a tiny opening of about 0.1 mm. between the valves of the mollusks was sufficient to permit the insertion of starfish stomach. The pulling of valves apart is probably repeated at intervals while the stomach remains partially compressed. The observations of Feder (1955) on *Pisaster ochraceus* show that this starfish can open its prey by force alone. Another Pacific Coast species, *Evasterias troschelii*, was found to exert a force in excess of 5,000 g. during an attack on artificial clams baited with *Mytilus* meat (Christensen, 1957). The fact that starfishes are able to open mollusks by force alone does not eliminate the possibility of an additional narcotizing effect produced by starfish secretion. The problem of how the starfish opens its prey has not yet been finally solved, although present evidence favors the mechanical hypothesis.

Not all starfishes feed by everting their stomachs and digesting the body of the victim without ingesting it. Many of them are scavengers feeding on dead animals found on bottoms while others are capable of catching and consuming live fishes. Many interesting cases of starfish attacks on various marine animals including fishes are described by Gudger (1933).

Starfish are usually found in water of high salinity and do not invade the oyster grounds in brackish waters. The salinity level between 16°/₀₀ and 18°/₀₀ below which *A. forbesi* cannot exist is a natural barrier to the distribution of this species. This conclusion is based on field observations along the Atlantic coast and on experiments at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn. (Loosanoff, 1945). In New England waters, starfish are controlled by mopping or dredging to remove them, and by dispersing calcium oxide and other chemicals to kill them or to make a protective chemical barrier around an oyster bed.

Flatworms

Turbellarians of the genus *Stylochus* and *Pseudostylochus*, commonly known as oyster leeches, are predators which attack adult and young mollusks and frequently inflict serious damage to oyster populations. In 1916 and 1917 attacks of *Stylochus* on oysters in Cedar Keys on the west coast of Florida killed about 30 percent and in one or two localities 90 percent of the adult oysters. The mortality of oysters in Apalachicola Bay, Fla., allegedly caused by the "leech," was investigated for the U.S. Bureau of Fisheries by Pearse and Wharton (1938), who could not state definitely that the distruction was due to *S. inimicus* Palombi[10] and suggested that the oysters were first weakened by some unknown cause and that *Stylochus* invaded those which were unable to protect themselves. *S. frontalis* tolerates water of low salinity (6°/₀₀), but according to Pearse and Wharton does not lay eggs in salinities less than 15°/₀₀.

S. ellipticus (Girard), found in Atlantic coastal waters and also reported from the Gulf (Hyman, 1939, 1954), lives among oysters, shells, barnacles, and rocks. The turbellarian was reported to destroy young oysters on the flats at Milford,

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[10] The identification was corrected by Hyman (1939) who found that the Florida leech belongs to the species *S. frontalis* Verrill.

Conn. (Loosanoff, 1956). Apparently it has no difficulty in entering oyster spat through the slightly opened valves. On the Pacific coast, the flatworm *Pseudostylochus ostreophagus* Hyman (Hyman, 1955) was reported to cause mortalities of from 6 to 42 percent among the imported Japanese seed oysters on various grounds. The worm bores keyhole perforations in the shells of young oysters (Woelke, 1957).

Crabs

Ryder (1884) was the first to include the blue crab, *Callinectes sapidus* Rathbun, and the common rock crab, *Cancer irroratus* Say, in the list of oyster enemies. He quoted complaints of oystermen working in Great South Bay, Long Island, N.Y., who stated that the crabs eat small oysters up to the size of a 25-cent coin and invade the the oyster planting grounds.

For many years crabs were not mentioned in oyster literature as potential enemies, but in the 1930's and 1940's there were reports from the U.S. Bureau of Fisheries Biological Laboratories at Milford, Conn., and at Pensacola (Gulf Breeze), Fla., that under certain conditions the blue crab, the rock crab, and the green crab, *Carcinides moenas* (Linneaus) destroyed oysters kept in outdoor tanks or placed in baskets with the crabs. Lunz (1947) reported that at Wadmalaw Island, S.C., the blue crab was probably the most serious pest in 1946 and destroyed more than 80 percent of the young oysters set on collectors. The crab's diet includes a great variety of food, including oysters. There is no evidence that they are attracted specifically by oysters, but it is apparent that they may destroy many small oysters in clusters by cracking their shells.

Mud prawns and fish

Brief mention should be made of the family Calianassidae (genera Upogebia and Callianassa), popularly known as "mud prawns" or "burrowing shrimps", which excavate deep burrows under oyster bed dikes. This activity drains water from the grounds, exposes the beds of *O. lurida*, and smothers the young oysters with material thrown up in burrowing (Stevens, 1928).

In the southern waters of the Atlantic coast, oyster beds are often invaded by schools of black drum, *Pogonias cromis* (Linnaeus), which feed on mollusks and occasionally cause extensive destruction of oysters, leaving behind piles of crushed shells. The fish uses its powerful pharyngeal teeth to crush the shells (fig. 398).

The diamond stingray of the Pacific coast, *Dasyatis dipterurus* (Jordan and Gilbert), also devours oysters, crushing them with powerful teeth. To ward off attacks by this fish, oyster grounds in California are surrounded by high fences, a practice used for the same purpose by French oystermen.

Birds

Various species of ducks are enemies of small *O. lurida* of the Puget Sound area. The extent of damage to oyster grounds near Olympia, Wash., was estimated in the fall of 1928 by the United States Biological Survey. McAtee, who conducted the field studies, reported (quoted from Galtsoff, 1929) that at that time 87 percent of the bluebills (*Nyroca marilla* and *N. affinis*) fed principally on oysters, which comprised 80.5 percent of the bulk of the food found in their stomachs. In 38 percent of white-winged scoters

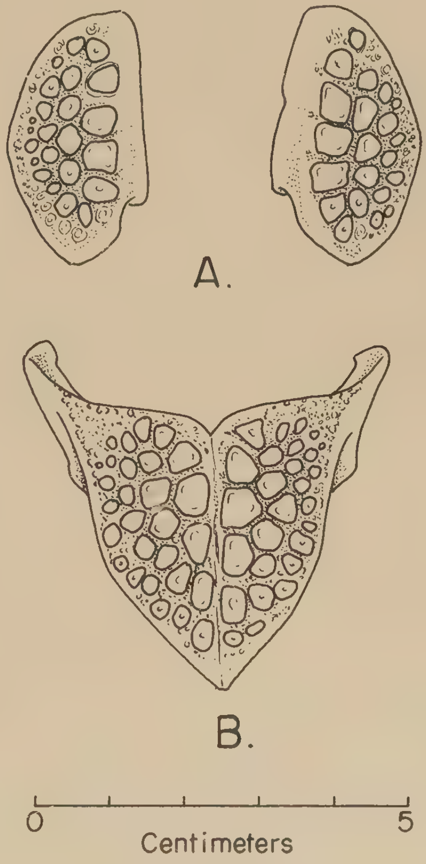

Figure 398.—Pharyngeal teeth of small size black drum *P. cromis*, used for crushing oyster shell. A—upper teeth; B—lower teeth.

(*Melamita deglandi*), about 70 percent of their stomach contents consisted of oysters. The number of birds in the Olympia Bay of Puget Sound during the 2-week period of daily observations (November 16–29, 1928) averaged 2,000. Together the three species of ducks were destroying about 8,000 oysters per day and causing material damage to the small oyster industry of the area.

The effect of predators on an oyster population can be evaluated by determining the percentage of oysters killed.

Man

Among the highly destructive predators of oysters, man occupies the most prominent position. Long before our era the stone age dwellers of the coast of Europe subsisted primarily on shellfish which they gathered from shallow water by wading and hand picking. The American Indians used oysters and clams for food, and dried and smoked shellfish meat for the food supplies which they took on their travels. On both continents numerous shell heaps or so-called kitchen middens dot the coastline and indicate the locations of primitive habitations or camp sites. A famous shell heap on the banks of the Damariscotta River, Maine, and many others are evidence of the former productivity of the oyster beds of past centuries. With the development of oyster fishing gear, man became able to gather oysters much more efficiently and extended his efforts to deeper water. Oyster dredges of various designs and dimensions remained for a long time the principal and very effective gear, until the appearance in the last quarter of a century of various mechanical suction pumps and other harvesters of much greater efficiency.

With the improvement of fishing methods, the oyster bottoms of the northern States became overfished and many were depleted. This was the fate of many oyster grounds along the shores of the Gulf of Maine, in New Hampshire, Massachusetts, and Rhode Island. In colonial times the earliest white settlers of New England feared the disappearance of their favored seafood and saw the necessity of protecting their shellfish resources by such legislative measures as restricting the size of catch and prohibiting the selling of oysters out of town. The results were ineffective, and many oyster bottoms, particularly in the northern part of New England, were destroyed.

The world's richest oyster bottoms in the Chesapeake Bay suffered a similar fate, but the depletion was more gradual and not as complete as in more northern waters. Regulations prohibited power dredging and set aside certain areas for the use of tongers only, but they were not sufficient to maintain the productivity of the oyster bottoms. The production of oysters continued to decline because of a general disregard of the basic conservation principle that the sustained yield of any renewable natural resource can be maintained only if the quantity removed does not exceed the quantity restored annually by reproduction and growth. Throughout the world the shellfish resources are depleted when more are taken than nature is able to replace.

Man must be regarded, therefore, as the most dangerous predator. On the other hand, through his action the productivity of an oyster bottom can be brought to the highest level. Since ancient times it has been known that oysters can be propagated and cultivated. The development of oyster culture in this country was particularly successful in the waters of Long Island Sound where the depleted shellfish resources were not only restored by oyster farming, but many thousands of acres of previously barren bottom were converted into productive farms under water. Thus, man as an ecological factor appears in a dual capacity—as a primitive destructor and as a progressive cultivator. Unfortunately, at present Long Island Sound is no longer a highly productive oyster farming area. The decline may be attributed to poor setting, low survival rate of young oysters, devastation caused by several hurricanes, and the high cost of farming operations.

At present the knowledge of oyster biology has advanced to such a level that effective methods can be employed both for sound management of natural, wild populations of oysters, and for development of highly productive farms for breeding selected strains of oysters. The continuous decline of oyster beds is due not to a lack of knowledge but to failure to apply it.

Aquatic resources of the tidal areas along the Atlantic and Gulf coasts of the United States are threatened by human activities other than overfishing. Many formerly productive areas of the coast have been damaged beyond reconstruction by the filling of marsh lands for industrial sites, by the construction of thruways, marinas, real estate developments, and trash and garbage disposal areas, by ever-increasing discharge of domestic sewage and trade wastes, and by numerous contaminants which reach natural waters as a result of widespread and nonselective use of insecticides and pesticides. Danger from the discharge of radioactive materials from nuclear plants and the disposal of low level radioactive wastes in the sea not far from shore presents a new and serious threat to the usefulness of the renewable aquatic resources of coastal areas.

Some of the changes produced by man such as improvement of coastal waters for navigation, construction of hurricane barriers, use of tidal land for building of industrial plants are consistent with rapid population growth and industrialization. Other changes, such as pollution, destruction of natural oyster beds by failure to return shells and other materials needed for the attachment of young oysters, and overfishing are unnecessary and should be avoided.

A balance between the needs associated with industrial progress and population pressure on one side, and effective conservation of natural aquatic resources on the other can and must be found.

POLLUTION

The pollution problem is complex. It has many facets that should be studied from social, economic, and biological points of view. An investigation of the biological aspects of pollution, discussed in this section, deals with the complex ecological relationship between the life in the tidal areas and the environment affected by the addition of a number of organic and inorganic contaminants.

One of the major difficulties encountered in studies of the biological effects of pollution is the lack of a generally accepted definition of the term. Pollution means different things to different people: to a Public Health officer pollution implies a potential health hazard caused by the discharge of domestic sewage and industrial waste; an engineer of a manufacturing plant is primarily concerned with the quality of water needed for the industry; the conservationist has in mind danger to wildlife and means for its protection; sport and commercial fishermen fear that foreign substances discharged into coastal waters will affect the availability of fish; a marine ecologist tries to find out how the animal and plant life is affected by changes in the environment; and the layman, considering that pollution is synonymous with filthy conditions on beaches and in coastal waters, raises his voice in protest against unsanitary and esthetically objectionable situations.

In court litigations involving damages allegedly caused by pollution, a biologist appearing as an expert for either side is handicapped in his testimony either by lack of a legal definition of pollution or by the generalities used to describe it. No definition of the term pollution is given in the Oil Pollution Acts of 1924 and 1961. The Water Pollution Control Acts of 1948 and 1961 (United States Congress, 1948, 1961) make frequent references to the "abatement of stream pollution" and declare in the 1948 act that pollution is a public nuisance "which endangers the health or welfare of persons in a State other than that in which the discharge originates." The inclusion of the word "welfare" puts emphasis on the economic aspects of pollution and, therefore, increases the scope of the definition.

After conducting a comprehensive study of all available State, Federal, and international pollution laws, the U.S. Public Health Service (1950) prepared the following broad definition of pollution:

> "Pollution" means such contamination, or other alteration of the physical, chemical or biological properties, of any waters of the State, or such discharge of any liquid, gaseous or solid substance into any waters of the State as will or is likely to create a nuisance or render such waters harmful or detrimental or injurious to public health, safety or welfare, or to domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or to livestock, wild animals, birds, fish or other aquatic life.

Although this definition is broad and useful, it has not been incorporated in existing Federal statutes and, therefore, lacks legal weight.

The amount of waste discharged into coastal waters of the United States from municipalities and industrial plants in the last decade has reached astronomical proportions and is being augmented by runoff water which carries the numerous organic phosphorus and hydrocarbon insecticides used in both control and eradication of agricultural crop-damaging pests. Under present conditions it is probably impossible to find water along our coast which has not been contaminated.

Some pollutants contain highly toxic substances and cause mortalities among marine populations. Others are less toxic and have no lethal effect on adult organisms but decrease the rate of survival of their larvae; decrease the rate of growth of juvenile forms and affect the reproductive capability of an organism. Sublethal concentrations of such poisons can also destroy one or several links of the food chain in the sea, and so affect the food supply for the population of animals or plants important for human welfare. The normal ecological environment may be so changed that some planktonic organisms, most useful to shellfish as food, disappear and are replaced by a luxurious growth of microorganisms not only useless but even harmful to water-filtering mollusks. Although great advances have been made in the technique of bioassays, the results of short-term tests lasting no longer than 72 hours are of little use in determining the effects of prolonged exposures of fish or shellfish to low concentrations of poison. Furthermore, since the criteria for the welfare of marine populations are not known, it is impossible to set requirements for purification of pollutants before they are permitted to be discharged into the sea. The Federal Water Pollution Control Act of 1961 authorizes the Secretary of Health, Education, and Welfare to organize comprehensive programs of investigation which in the course of years will solve many of the existing pollution problems.

Detailed descriptions of all types of pollution that may affect the productivity of oyster bottoms and methods of their detection and control are beyond the scope of the present chapter, which is limited to a discussion of the general principles applicable to the majority of situations and to a description of the most important types of pollutants encountered on oyster bottoms. Bibliographical references listed at the end of the chapter are limited to the more pertinent papers. Discussions of more specialized pollution problems are listed in a bibliography prepared by Ingram (1957) and also appear in papers published in Tarzwell (1957, 1960).

The production of oysters in the United States is declining at a rapid rate (Galtsoff, 1956). As a sedentary animal devoid of any means of locomotion after setting, the oyster is vulnerable to environmental changes which weaken it and make it less resistant to infection. Under natural conditions unspoiled by human activities, the oyster is in an equilibrium with its environment; this adjustment, which may be called a steady state, is the result of thousands of years of adaptation and natural selection. It may be upset by the sudden presence of materials not normally found in sea water or by excesses or deficiencies of its normal components.

Two types of pollution are commonly found on oyster grounds: domestic sewage and trade wastes. In natural waters both types of pollutants undergo gradual changes which lead to a degree of purification, but at the same time deposit sediments that cover oyster beds and change the character of the bottom. Natural purification is not effective, however, in the case of detergents and radioactive waste, which constitute a growing menace to the safety and purity of our coastal waters.

Domestic sewage

Contamination of water by domestic sewage is the oldest type of pollution; it probably began during prehistoric times when man settled on the shores of the rivers and bays and used natural waters as the easiest and most convenient way of disposing of the excrements and unwanted waste. The problem has reached enormous proportions with the population growth and the necessity of disposing of quantities of domestic sewage in an organized manner.

The discharge of untreated domestic waste has a threefold effect. It covers the bottom with a sludge which smothers the oyster bed, affects the normal functions of mollusks by reducing the oxygen content of the water, and at the same time greatly increases the bacterial content of the water. Oysters, in common with other water-filtering mollusks, retain and accumulate these bacteria in their bodies. The degree of pollution is determined by the abundance of *Escherichia coli* found in the water. The bacterium itself is not pathogenic, but is used as an index of pollution. Procedures for determining the abundance of *E. coli*, the so-called MPN (most probable number), are described in great detail in Jensen (1959). They are strictly followed by State and Federal Public Health Officers and other officials responsible for certifying grounds from which shellfish may be harvested for human consumption. Areas in which the MPN of *E. coli* exceed the permissible maximum of 70 per 100 ml. are condemned and cannot be used for harvesting, but under certain specified conditions the polluted oysters and clams can be taken for planting to an unpolluted area. The presence of *E. coli* above the prescribed MPN eliminates the utilization of grounds for commercial fishery, but does not affect the survival and growth of the oyster population.

Industrial waste

The most common industrial pollutants entering oyster-producing areas stem primarily from the following industries: oil; paper; steel; chemicals; paints; plastics; leather; and food. The character of industrial waste varies with the product.

Because of the increase in the number of oil burning ships and the necessity of transporting crude oil in huge tankers that occasionally break and spill their cargo, oil pollution of the open sea has become a difficult international problem. Although federal and state laws forbid the discharge of oil into coastal waters, many of the bays and harbors of the United States are heavily polluted by oil. Through surface tension oil spilled on the surface of water spreads rapidly into a thin film or oil slick. In muddy waters suspended particles of clay and sand absorb oil, coalesce, and gradually sink to the bottom. In shallow waters oil laden sediment is disturbed by waves, and an oil slick reappears on the surface, sometimes considerable distances from the source of pollution. The absence of an oil slick is not, therefore, a reliable sign that water is not polluted. Crude oil absorbed by sediments retains its toxicity to oysters and other organisms for a considerable time (Chipman and Galtsoff, 1949).

With the expansion of the pulp and paper industry along the Atlantic and Pacific Coasts, pollution of coastal waters by red and black liquors, the waste products of this industry, became serious. Both types of waste contain toxic substances which adversely affect oyster physiology. As in other types of pollution, the discarded material is usually oxidizable and has high oxygen demand. It is, however, only in extreme instances of gross pollution that the oxygen content of the water is lowered to the point that it suppresses the principal physiological functions. Poisons, present in trade waste, are more dangerous than the high oxygen demand because they directly affect the function of the various organs. In spite of great variety in the composition of trade wastes their toxic effect can be demonstrated by constructing a toxicity curve which shows how the pollutant depresses the function that was selected for testing. An oyster heart preparation (see ch. XI, p. 247) can be used conveniently because of the great sensitivity of the heart muscle to many poisons and drugs. Another measurable function is the transport of water by the gills for feeding, respiration, and discharge of excreta. This function ceases when the valves are closed. The presence of pulp mill pollutants reduces the number of hours the valves are open in proportion to the concentration of toxic substances in the water. Under normal conditions and at temperatures of 60° to 70° F. oysters remain open on an average of 20 to 22 hours a day. If the logarithm of concentration of black liquor or crude oil extract is plotted against the number of hours closed, the relationship can be expressed by a straight line as shown in fig. 399. Toxic substances of pulp mill effluents and the extracts of crude oil affect the frequency of ciliary beat and so interfere with the coordination of ciliary motion with the result that the pumping capacity of the gills is reduced. The reduction is proportional to the concentration of physiologically active materials (fig. 400). This type of relationship was found in studies on the pollution of oysters by red and black liquor and by water soluble components of crude oil (Galtsoff, 1931b; Galtsoff, Chipman, Engle, and Calderwood, 1947; Chipman and Galtsoff, 1949). The observations on crude oil are in agreement with data reported

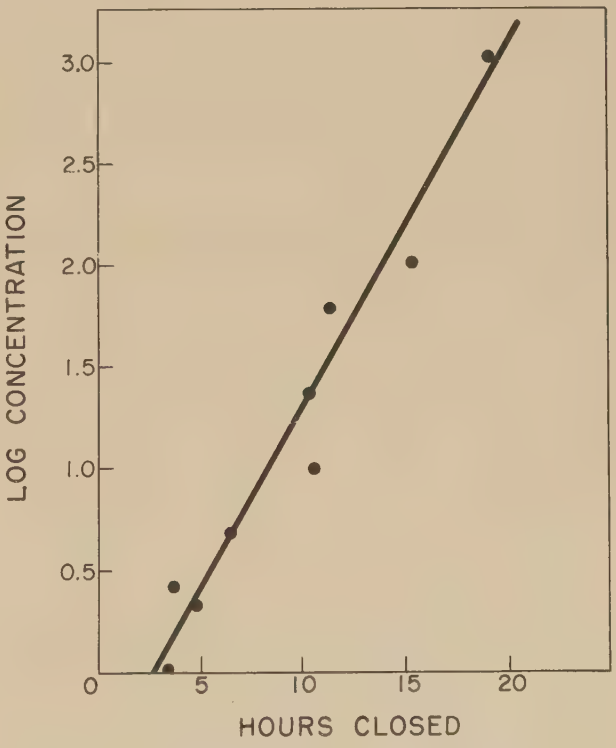

FIGURE 399.—Effect of concentration of pulp mill effluent discharged into the York River on the number of hours oysters are closed during every 24-hour period. From Galtsoff, Chipman, Engle, and Calderwood, 1947.

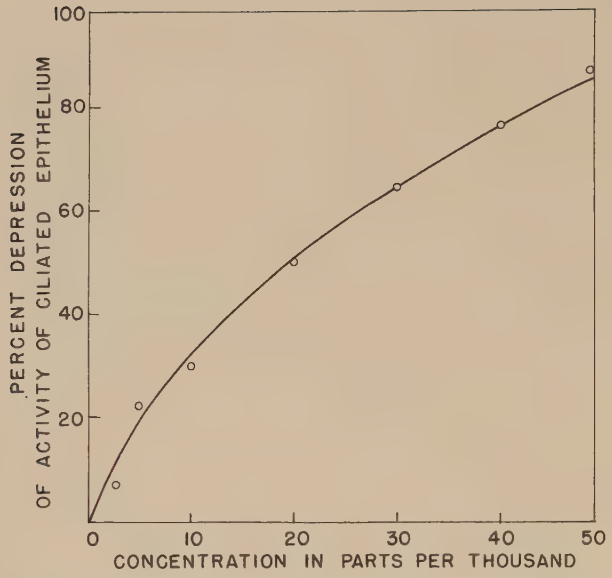

Figure 400.—Depression of the activity of ciliated epithelium of oyster gill by increased concentration of pulp mill effluent (black liquor) of specific gravity 1.0028. From Galtsoff, Chipman, Engle, and Calderwood, 1947.

by other investigators (Seydel, 1913; Veselov, 1948) on the toxicity of crude oil to fishes.

Determination of the toxicity of some pollutants is difficult because they may be present in such low concentrations that they are near or below the threshold of sensitivity to chemical methods. Their presence in even minimal quantities should be considered potentially dangerous to sedentary animals unable to avoid them. Another detection problem is that in many industrial plants the discharge of effluents is not continuous but is frequently interrupted or made during the night and early hours of the morning. Pollution studies must include taking composite samples of water with automatic samplers over a period of several hours. Some contaminants are unstable; after being discharged into sea water they are gradually oxidized, precipitated, neutralized, and become less harmful. The rate of this self-purification of water depends on many conditions, temperature, salinity gradient, sedimentation, and currents. To avoid inconsistencies in results, toxicity tests with such materials should be carried out only with stabilized samples (Odlaug, 1949).

Bioassays made within a few days indicate the presence or absence in water of a physiologically active substance but do not determine whether the pollution is lethal to the animal. Long-term field and laboratory observations are needed to determine the lethal effects of a low concentration of pollutants.

Ecological studies in polluted waters show that under certain conditions the normal environment may be modified by the contaminant and become unsuitable for growth and reproduction of oysters. Pollution of Shelton Bay, Puget Sound, Wash., with red liquor discharged by a local pulp mill boosted the production of the diatom *Melosira* sp. to such an extent that the beds of *O. lurida* in the bay became covered with a thick layer of this fouling plant. A similar effect occurred in laboratory tests with red liquor made by Odlaug (1949). Oysters affected by red liquor were useless because of their poor quality and poor taste; their reproduction stopped completely. Normal conditions were restored after discharge of the pollutant was discontinued (McKernan, Tartar, and Tollefson, 1949).

The biologist who studies pollution of natural water should remember that there is no harmless pollution. All types of pollution are harmful to marine populations; only the degree of their effects differs. Frequently it is claimed that the enrichment of sea water by phosphates, nitrates, carbohydrates, and other organic matter is beneficial and will tend to increase productivity. In the case of water pollution by duck farms in Moriches Bay, Long Island, N.Y., indiscriminate pollution by duck manure caused an imbalance of nutrient salts and boosted the outbreak of microorganisms which had an adverse effect on shellfish (Redfield, 1952). Useful enrichment of sea water can be achieved only by controlled and balanced fertilization.

Oxidation is important in reducing or destroying the toxicity of certain contaminants of sea water (Galtsoff, Chipman, Engle, and Calderwood, 1947). The efficiency of oxidation is influenced by temperature and by the manner in which the pollutant is added to the water. Preliminary storage in tanks is helpful in removing objectionable solids, and cascading the effluent from storage tanks to the place of discharge will expedite its oxidation. The U.S. Public Health Service found that 10,400 factory outlets in 1950 were pouring their waste into natural waters of the United States; only 657 of them had waste treatment plants of adequate capacities. In about 30 percent of the plants, the method of treatment was unsatisfactory. The number of plants which at present discharge their waste into coastal waters and the amount of waste are not known.

Radioactive waste

The disposal of radioactive waste in the sea presents a new threat to shellfish resources; the concentration of radioactive materials in the bodies of water-filtering mollusks may render them unsafe for human consumption. Chipman (1960) showed that many of the radionuclides added to sea water become associated with both living and nonliving particles suspended in water. Experiments at the Radiobiological Laboratory of the Bureau of Commercial Fisheries at Beaufort, N.C. (Chipman, Rice, and Price, 1958; Rice and Willis, 1959), indicated that nearly all fission product radionuclides, and also those of the trace metals, that are added to algal cultures associate with marine plankton used by shellfish. If continuously available, radioactive particles may accumulate in filtering organs, on the body surface, and in the digestive tract of oysters and other shellfish.

The accumulation of radioactive pollutants in coastal waters is likely to become higher than it is at present if the current practice of dumping radioactive wastes from nuclear plants and many research institutions close to shore or in the lower parts of a river (Columbia River) continues indefinitely. This unwelcome possibility must be watched carefully, and a great deal of research remains to be done before a clear picture emerges of the potential dangers associated with the disposal of low level radioactive waste and the contamination of our fisheries resources.

To evaluate the effect of pollution on the productivity of oyster bottoms the following data are needed: the type and extent of pollution in relation to the total volume and movements of water in an estuary; the stability of the pollutant; its physiological action; the effect of long-continued exposure of oysters to low concentrations; and the determination of the lethal concentration of a pollutant killing 50 percent of a population, the so-called LD 50.

COMBINED EFFECT OF ENVIRONMENTAL FACTORS

Known effects of any single factor of the environment do not give a true picture of the situation found in nature. Factors of the environment always act jointly. One serious weakness of many ecological studies of marine populations is the tendency to correlate the results of biological observations with one or possibly two selected factors of the environment, such as temperature, salinity, or hydrography, and to disregard the effect of others. In reality, any factor can exert its effect only in conjunction with others. It is impossible to separate the effect of chemical changes caused by a pollutant from the movements of water and from the effects of the pollutant on the food chain. Changes in the character of a bottom brought about by sedimentation cannot be separated from changes in sea water chemistry, or the food chain. An increase in the salinity of water encourages the invasion of grounds by some competitors and predators, while lowered salinity forms a barrier to inroads by starfishes and drills.

The combined action of several factors produces a far greater effect than that caused by any single factor. Findings of what effects combined factors have on agricultural plants (Rübel, 1935) are fully applicable to conditions affecting aquatic animals. So far, however, no adequate studies have been made on the problem of measuring the joint effect of several factors of aquatic environment. The relationship of all factors probably can be expressed by a very complex formula of the type developed by Riley (1947) for seasonal fluctuations of phytoplankton populations in New England coastal waters. The very complexity of a formula of this type precludes its usefulness for the practical purpose of evaluating conditions on oyster bottoms. The oyster biologist is often confronted with the necessity of expressing his opinion on the quality of the oyster beds. His impression is given in general, nonspecific terms as adequate, good, very good, marginal, etc., which do not disclose the reasons for a particular evaluation.

My proposed method of scoring eliminates the uncertainty of personal impressions and assigns to each factor a value which indicates the degree of its effectiveness on a given population of oysters. The method has been applied successfully in the evaluation of oyster bottoms in New England, the south Atlantic coast, and in some Gulf States (Galtsoff, 1959). It has been already stated above (p. 399) that the optimal condition of existence with reference to a single positive factor can be assigned the numerical value of 10. Degrees of inadequacies are given numerical values in descending numbers from nine to one. Negative factors are treated in much the same way. Complete absence of a negative factor refers to optimal conditions, and therefore, is designated zero, while the degrees by which the factor adversely affects an oyster population are assigned the numbers diminishing from nine, for 90 percent of negative influence, to one, which denotes 10 percent or less of harmful effect. The zero value of a positive and 10 value of a negative factor are omitted because under the proposed system such values denote complete unsuitability of environment for the existence of an oyster population.

A combination of environmental conditions which determine the productivity of an oyster bottom is summarized in simple tabular form by listing in two separate columns all positive (+) and all negative (—) factors and assigning to them their rank. As an example of the method, the data for one of the highly productive areas in the northern Cape Cod area, where observations were made for several years, are presented in table 46. In this area, which approaches ideal conditions, the presence of predators is the only serious problem.

The overall evaluation is made by summing up all positive factors, Σf^+ and all negative factors, Σf^- and by deducting the sum of the negative factors from the sum of the positive. Under this system the highest score of 50 refers to a theoretical situation where all positive factors are optimal and negative factors are absent. The low score of 10 and less refers to marginal conditions. Tabulation of factors is of great practical advantage because it shows at a glance the causes of low productivity and how it can be improved. The following tabulation shows the scores that in my opinion apply to various degrees of productiveness of oyster bottoms:

Excellent	41–50
Good	31–40
Average	21–30
Poor	11–20
Marginal	10 and less.

Table 46.—*Evaluation of the productiveness of an oyster ground in the northern part of Cape Cod*

Positive factors (+)	Score	Negative factors (—)	Score
Bottom	10	Sedimentation	----
Water movement	10	Disease	----
Temperature	5	Competition	2
Water quality	10	Predation	5
Food	10	Pollution	----
Total	45	Total	7

Note.—Overall score 45—7=38; ---- in negative score indicates absence of a factor.

In its present form, the method obviously oversimplifies the problem because it considers all the factors as equally significant, which may not be true. The present lack of understanding of the interaction within a complex ecological system bars expression of this interrelation in a more precise form. Growing interest in studies of the sea and its resources, however, gives promise of rapid progress in determining the intricate relationships among the principal factors that govern the prosperity of marine populations. The resulting knowledge will provide the basic data for designing effective methods of utilization and conservation of the renewable resources of the sea.

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